STUDIES ON MICROBIAL DYNAMIS ON COTTON IN AND AROUND THE SPINNING MILL JHANSI

THIS THESIS SUBMITTED TO THE
UNIVERSITY OF BUNDELKHAND
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
(BOTANY)

BY

SHIKHA RAIZADA

MICRO BIOLOGY STUDY CENTRE
DEPARTMENT OF BOTANY
BIPIN BIHARI POST GRADUATE COLLEGE
JHANSI - 284001

1992

ACKNOWLEDGEMENT

I am overwhelmed at this opportunity of expressing my profound gratitude and indibtness to my guide Dr. M.C. Kanchan, M.Sc., PH.D., Head of the Botany Department, Bipin Bihari Post Graduate College, Jhansi (U.P.). His keen interest, unflagging guidance, constant encouragement and invaluable advice has enabled me to present this work.

I feel obliged and offer my sincere thanks to Dr. S.C. Shrotri, Principal, Bipin Bihari Post Graduate College, Jhansi for his untiring interest, valuable suggestions and for providing all the laboratory facilities.

My sincere thanks are also due to Dr. K.S.Johri, Department of microbiology, Pusa Reaserch Institute, Delhi and Dr. A.K. Agarwal, Medical College, Jhansi, for their valuable suggestions and help for providing laboratory facilities. I am also thankful to Shri J.B.Malhotra, Chief Executive, U.P.STATE Spinning mill, Jhansi & Shri R.K.Dhimman, Labour Officer, Spinning mill, Jhansi, for their Co-operation and providing facilities in collecting the samples.

My sincere thanks are also due to lecturars, Colleagues and friends of the College for their co-orperation , advise and sparing facilities time to time

ACKNOWLEDGEMENT

I am overwhelmed at this opportunity of expressing my profound gratitude and indibtness to my guide Dr. M.C. Kanchan, M.Sc., PH.D., Head of the Botany Department, Bipin Bihari Post Graduate College, Jhansi (U.P.). His keen interest, unflagging guidance, constant encouragement and invaluable advice has enabled me to present this work.

I feel obliged and offer my sincere thanks to Dr. S.C. Shrotri, Principal, Bipin Bihari Post Graduate College, Jhansi for his untiring interest, valuable suggestions and for providing all the laboratory facilities.

My sincere thanks are also due to Dr. K.S.Johri, Department of microbiology, Pusa Reaserch Institute, Delhi and Dr. A.K. Agarwal, Medical College, Jhansi, for their valuable suggestions and help for providing laboratory facilities. I am also thankful to Shri J.B.Malhotra, Chief Executive, U.P.STATE Spinning mill, Jhansi & Shri R.K.Dhimman, Labour Officer, Spinning mill, Jhansi, for their Co-operation and providing facilities in collecting the samples.

My sincere thanks are also due to lecturars, Colleagues and friends of the College for their co-orperation , advise and sparing facilities time to time

when ever required.

In submitting this thesis, I owe much to my Parents, my mother-in low, father-in-low & my husband for their sustained interest and for keeping me aloof from domestic worries.

Shikha Raizada
(nee-Saxena)

DECLARATION

I hereby declare that with the axception of the guidance and suggestions received from my supervisor, Dr.Munish Chandra Kanchan, M.Sc.,Ph.D. Head, Department of Botany , Bipin Bihari Post -Graduate College, Jhansi (U.P.) , this is my original piece of work carried out in microbiology study centre, Department of Botany, Bipin Bihari Post Graduate College, Jhansi, for the degree of Doctor of Philosophy.

Shikha Raizada

Shikha Raizada(nee Saxena)

GENERAL NOTE

"$\mathcal{U}$ is written in text as u, thus it should be read as $\mathcal{U}$.

Dr. M.C.Kancan, M.Sc.Ph.D.
HEAD
Department of Botany
Bipin Bihari Post Graduate College
J H A N S I

Dated : 17-9-'92.

SUPERVISOR'S CERTIFICATE

I hereby certify that this thesis entitled "Studies on microbial dynamism on cotton in and around the spinning mill Jhansi" is an original piece of research work carried out by Mrs. Shikha Raizada (nee Saxena) under my guidance and supervision for the degree of Doctor of Philosophy of university of Bundel Khand ,Jhansi, U.P.

(DR. M. C. Kanchan)

CONTENTS

CHAPTER		PAGE
	ACKNOWLEDGMENTS	i
	DECLARATION	iii
	GENERAL NOTE	iv
	SECTION - I	
	GENERAL	
1.	Gernral Introduction.	1
2.	Climate of Jhansi during the course of study.	11
	SECTION - II	
	MICROBIAL DYNAMISM	
3.	General Introduction.	14
	SUB SECTION - A	
	QUANTITATIVE AND QUALITATIVE STUDIES	
4.	Introductions.	18
5.	Experimental -Isolation of micro-organisms.	24
6.	Result and conclusions.	30
	SUB SECTION - B	
	COMPARATIVE CELLULOLTIC ABILITY	
7.	Introduction.	47
8.	Experimental.	51
9.	Result and conclusions.	53
	SUB SECTION - C	
	COMPETITIVE SAPROPHYTIC COLONIZATION	
10.	Introduction.	54
11.	Experimental.	60
12.	Result and conclusions.	63
13.	Summary and discussion.	65

SECTION - III

CONTROL MEASURE

14. Intoduction. 81

15. Experimental. 89

Exp. A :-Preliminary screening of selcted organism against fungicides and antibiotics.

Exp. B :-To study the minimum inhibitory concentration of fungicides and antibiotics against the test organisms.

Exp. C :- To study the effect of selected fungicides and antibiotics on radial growth of test micro-organisms.

Exp. D :- To study the effect of fungicides on mycelial mat of fungal test organisms.

Exp. E :- To study the effect of fungicides and antibiotics on spore germination of micro-organisms.

16. Result and Conclusions. 101

17. Summary and discussion. 116

SECTION - IV

ENZYMOLOGICAL STUDIES

18. Introduction. 132

19. Experimental. 140

20. Result and conclusion. 146

21. Summary and discussion. 153

SECTION - V

22. General summary and conclusions. 159

SECTION - VI

23. Miscellaneous. 176

A. Biblilography.

B. Appendix I and II.

C. Plates.

SECTION I

GENERAL

CHAPTER - 1

GENERAL INTRODUCTION

The U.P. State spinning mill is situated on Jhansi Gwalior Road adjoining the air field. The mill apart from having two working units have residential plots for the workers and executives both. About 5000 workers are involved in the day and night shifts. They work in different chambers of the functional unit in which temperature and humidity is maintained according to the requirement to spun cotton into threads.

The bales of cotton arriving at the mill are handled by a number of workers. During processing cotton, fibers might get sprayed with pathogenic or nonpathogenic organism. These may multiply or remain viable as the temperature and humidity maintained within the mill is quite suitable. The small fibres or lint floating in the air are being constantly trapped in underground tunnels by suction, but still quite a good amount of fibres floats in the air. These fibres may act as vactors for passing the organism from one place to another.

The organism during such colonization are important as they might be involved in deterioration of cotton or in causing infection to the workers. Around the working units of the mill some cotton floats in the atmosphere and finally settles on the ground, telephone

poles, electric wires and fences etc. These builds up their own ecological niche and host a number of microbes adding to the pollution of the atmosphere and the organisms sprayed during handling with in the mill adding to the pollution of the atmosphere. To understand microbial dynamism it is necessary to make a successive quantitative and qualitative study of the microflora involved.

Some Toxigenic fungi on cotton have been reported to occur by Diener et. al., (1976),Nigam et. al., (1960); Bagga (1970); Lgreere (1973); Simbya et. al., (1969); Ashworth et. al., (1971); Ahmad and Gulatia (1943). During last 60 years a number of techniques have been developed for the isolation and study of the fungal flora by some workers Warcup (1960, 1967) Parkinson et. al., (1971); Johnson and curl (1972).

There are many reports on the capacity of micro organism to utilise cellulose in-vitro. Garrett (1962 , 1963 & 1966); Hogg (1966); Reese and Lavinson (1952); Rai (1970); Dwivedi and Singh (1974); have suggested that the successfull saprophytic colonization of fungus largely depends upon its Cellulolytic ability. This is reflected by the amount of cellulose utilized by the organism. The ability to utilize cellulose is considered to be essential for the

existance of saprophytic fungi by Malin (1948); Siu (1951) has given a list of cellulolytic fungi isolated from competitive colonizing conditions of cotton fibres. Competitive saprophytic colonization is the invasion of fibres in competition with other saprophytic organism, Garrett (1944 , 1962) defined this as the Summation of physiological activity brought about during the succession in colonization of dead matters. The physiological character that helps are the fast growth rate, rapid spore germination, good enzyme production, production of antibiotic substances and tolerance of antibiotics produced by others saprophytic colonizers. Garrett (1963b) suggested that in colonization of dead organic matters the competitive saprophytic ability is one of the main factor, others are inoculum potential and environmental conditions. The success of a fungus during competitive saprophytic colonization of cotton depends on its intrinsic biochemical ability to exploit or decompose or utilize that particular substrate.

Competitive saprophytic ability deserves more attention because this is one of the genetically determined characteristics of the microorganism. Number of techniques for estimating competitive saprophytic colonization has been given by Butler (1953A); Rao(1958); Wastie (1961); Butler (1953 a,b,c); Macer

(1961); Sadasivan (1939); etc.

Fungicides and antibiotics are widely used to control the growth of various disease producing organism, but their role in preservation and storage of fibres are less known. Most of the fungicides cause problems to the humans during handling these fibres and thus unsafe to be used frequently at such places. The use of fungicides/antibiotics exert a positive or negative influence on the total microflora, intraction of micro-community with the fungicides has been discussed by Cole and Turgeon (1978), Kuthubutheen and Pugh (1979), Rai and Shrivastava (1982) and others. Such substances will inhibit the microbes by either inhibiting cell wall formation or by damaging the cytoplasmic membrane or interfere with protein synthesis or, inhibit nucleie acid metabolism at the cellular level. At the molecular level they interfere with enzyme synthesis. These changes some times cause unexpected Problems Domsch (1964); Lockwood (1964); New Hall (1955) and collins (1965), therefore the use of chemicals should only be recommended in cases when they are absolutely necessary inhabitation of fungi is the underlying principal of almost all fungicides, effectiveness of fungicides depends upon the quantity, nature and age of inoculum as well as pH and concentration of the medium. Donald (1972); Domsch

(1964) suggested that action of any fungicides is comperatively low at low temperature. Humidity may effects both the penetration of chemical and also degree of sensitivity of the fungus.

Antibiotics are also widely used against microorganism. These are antimicrobial agent of microbial origin that can inhibit the growth of bacteria or even destroy them and other microorganism. To determine the effectiveness of various antibiotics, senstivity test are performed against various microbial species.

The main contituents of cotton fibre is cellulose while only a very small fraction is the pectic substance, hence celluloytic enzymes will have a major role in colonization or deterioration. In other Words the growth of organisms on these substances will depend upon its enzyme system, Enzymes are produced extra cellularly which degrade the cellulose, resulting into its hydrolysis. The enzyme involved is cellulose and fungi producing them are known to be cellulolytic. Cellulolytic activity has been worked out in detail by Reese (1946,1957); Garrett (1962); Mandel and Wabler (1969); Agrawal (1969); Bellamy (1974); Crawford and crawford (1976). Most of them found that cellulose is produced when organism are grown on substrates having cellulose. Filter papers,

cellophane paper and other materials like whatmans filter paper have been used by various workers to produce cellulose. Tribe (1957); Wabley and Duff (1962); Pugh (1966) and some others used even cotton and leaf litter Agrawal (1969); Pandey (1980) to observe cellulose activity, most of them suggest that cellulose exists in C_1 and C_x form, (C_1) degrading the native cellulose, while the others (C_x) degrading the product produced by C_1 enzyme. The C_x complex hydrolysing B-1, 4-glacosidic bond does not act on crystaline cellulose in the absence of C_1 enzyme. The basic concept remains that the native cellulose degradation involves two types of enzyme C_1 and C_x (Oso, 1978).

Besides cellulose, pectic substances are also found in the plant cells and play a vital role, in forming the inner cellular cement. This material is also degraded by micro-organism called pectolytic. The pectic enzymes involved in tissue degradation are pectine methyl esterase, pectinmethyl galacturonase, polygalacturonase and transeliminase. The production of pectolytic and cellulolytic enzymes have been reviewed by Bateman and Miller (1966); Wood (1955, 1960, 1972); they have also discussed the classification of these enzymes. Norkran (1963); Reese (1963); Furgus (1969); Whitker (1971), and Goel and Mehrotra (1973-1974)have

studied the effect of some fungicides and antibiotics on pectolytic and cellulolytic enzyme activity. They have found that these substances have an altering effect on the enzyme production. Hence the study of these enzyme system is of utmost importance in both deterioration and colonization of cotton fibres.

Considering the human population involved in operating the mill, the cotton fibres being colonized by micro-organism while floating or deteriorating and the hazard these organism may cause, it is extremly important to work out the periodic colonizaion of the microflora, their capability of cellulose utlization, competitive colonization, their relative engyme production and the effect of antibiotic fungicide on the microorganisms involved, with these considerations the study has been planned in the following lines :-

Section I CLIMATIC VARIATION

This section deals with the general climatic variation in and adjoining area of the spinning mill. These observations were conducted for the entire study period. Rainfall, temperature and humidity will be the main parameters under consideration

Section II : MICROBIAL - COLONIZATION

A- Quantitative and Qualitative Dynamism of Micro-organisms

This subsection deals with the

collection of cotton samples from various places i.e. the store room, floor of the spinning chambers and underground channels within the spinning mill. From these samples microorganism were isolated and analysed both quantitatively and qualitativly at the interval of 15 days for one year so as to find out the absolute number and percentage colonization and frequency during the various seasons of the year. In addition to these, microorganisms inside & outside the mill were also isolated from air.

B-Comparative Cellulolytic ability

This subsection deals with the studies on comparative cellulolytic ability of the dominating and most frequently found isolated in subsection "A".

The method employed is that of Garrett (1962) and the composition of the medium employed is of Hogg (1954) with the modifications that filter paper is replaced with cotton as the sole carbon source. Only the fungi which dominated during the study period in subsection "A" or fungi with special significance were selected for the peresent investigations.

C-Competitive Saprophytic Colonization

This subsection deals with the competitive saprophytic colonization of the mycoflora selected in

subsection "B". The method employed is that of Wasties (1961).

Section III Control Measures

In this section antibiotic fungicides available in the market were screened to study their inhibitory effect against the organism selectd from section II. Effects on the radial growth, mycelial mat and spore germination were analysed in its different dilutions, so as to find out their minimum inhibitory concentration (MIC). This enables us to list out the fungicides / antibiotic in their MIC against the micro-organism so that if required they could be recommended for spray on cotton inside the mill along with the spraying water.

Section IV Enzymological studies

The organisms selected from section II. Were studied for their enzyme production capabilities. These were studied in vitro at different stages of the organisms growth on broth cultures with cotton as the sole carbon source. For this study both cellulolytic and pectolytic enzymes were studied but main attention was towards cellulolytic enzymes, as the fibres contain about 94% cellulose. Enzymatic activity were also analysed by using MIC of the effective fungicides in the medium.

Section V General summary and conclusions

This section deals with the general summary and conclusions obtained from the entire study.

Section VI Miscellaneous

(I) Biblilography

(II) Appendix

(III) Plates.

CHAPTER - II

CLIMATE OF JHANSI

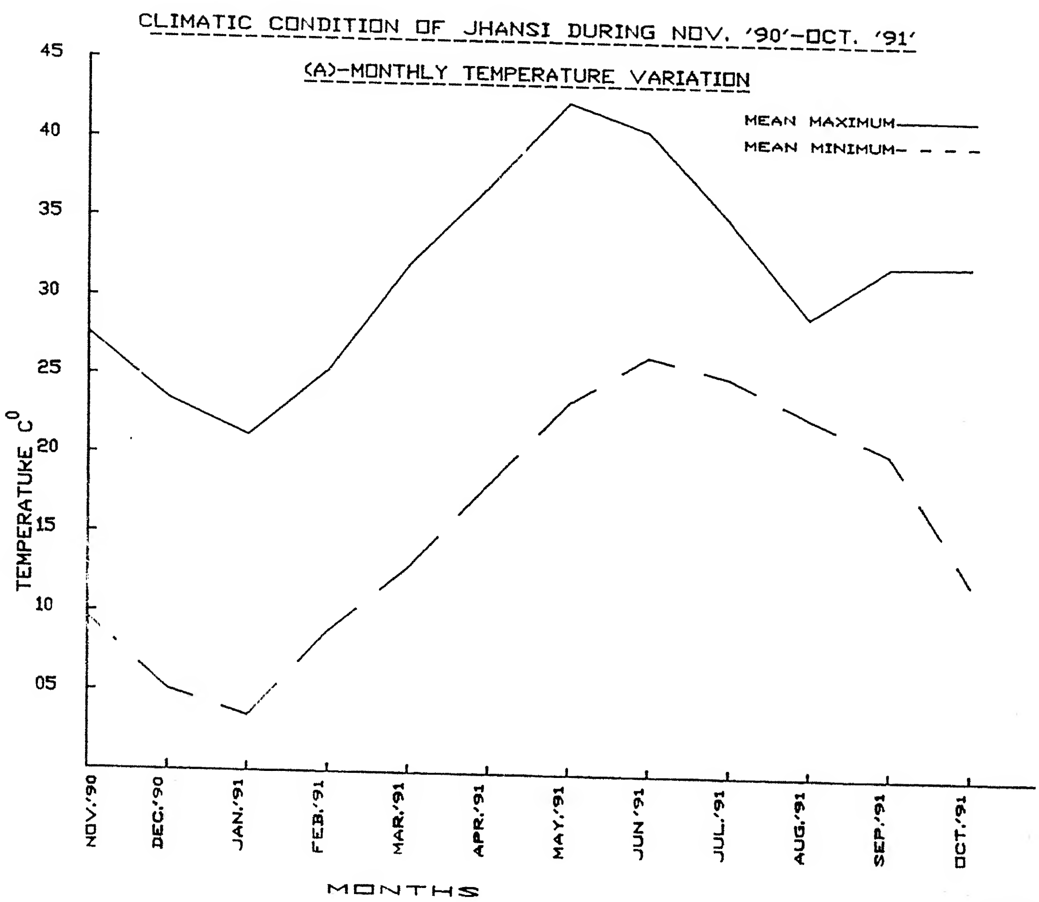
Jhansi is situated at a latitude of 25 degree 27' N and at longitude of 78 degree 35'E. The altitude is about 271 metre above mean sea level. The climatic conditions of Jhansi have been described by Shankarnarayan and Dabadghao (1970); Gupta (1976) and Trivedi (1975). On the basis of distribution of rainfall and variation in temperature (Table-II), climate of Jhansi can be said to be typically monsoonic and can be divided into three distinct seasons viz. rainy, winter and summer seasons.

The climatic data's for the study period are given in Table I and figure I. Mean minimum temperature varied from 4.5 degree C (January 1991) to 27.3 degree C (June 1991) and mean maximum temperature varied from 22.2 degree C (January 1991) to 43.5 degree C (May 1991) Relative humidity was minimum during may 1991 (23.5%) and maximum during August 1991 (79.0%). The average total annual rainfall recorded was 945.00 mm and it was maximum during July 1991 (460.3 mm). The monthly distribution of rainfall and number of rainy days for the study period are shown in Table I and figure I (C). On the basis of climatic record of the last fifteen years, the average annual temperature recorded was 26.5 degree C with mean minimum

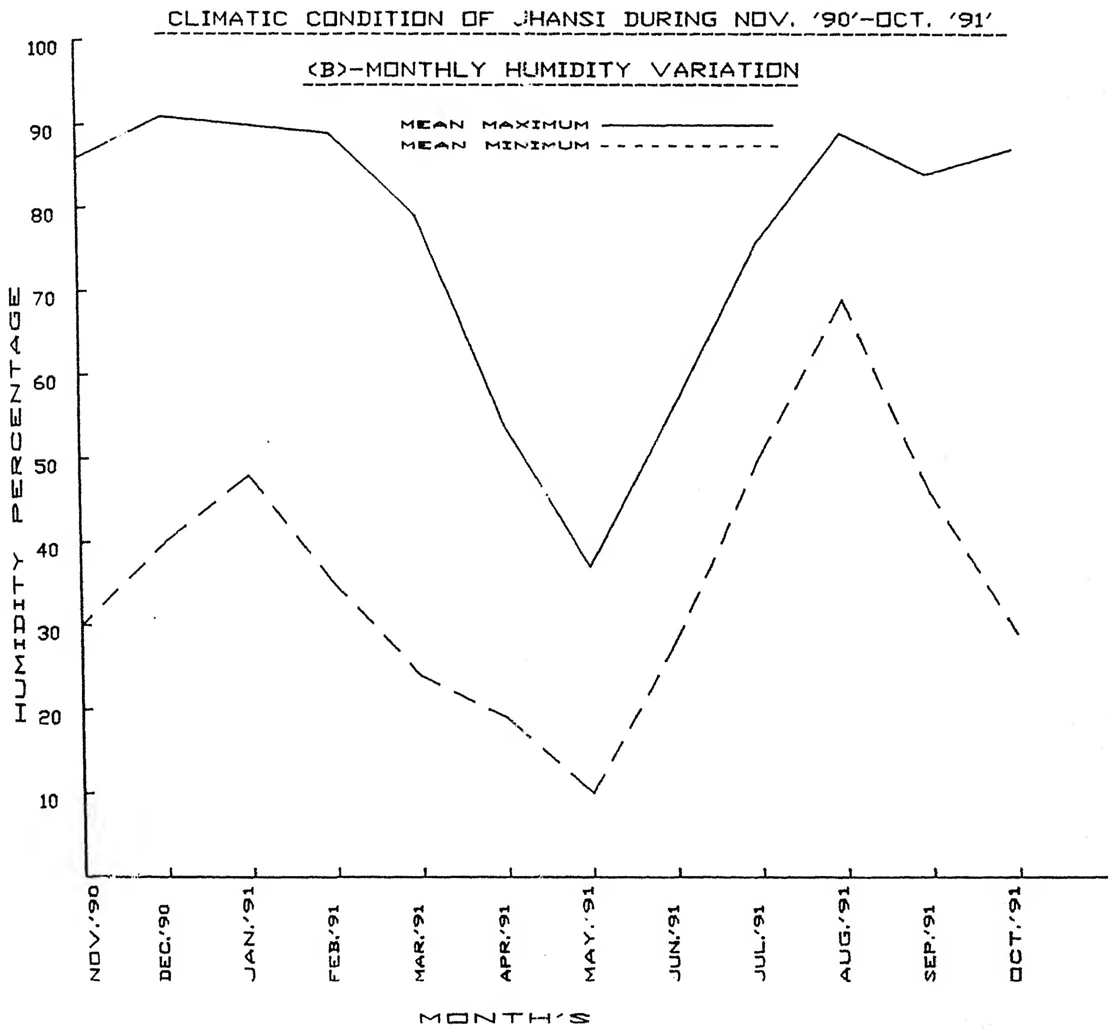
TABLE - I

CLIMATIC RECORD OF JHANSI OF THE STUDY PERIOD
(NOV.'1990 TO OCT.'1991)

MONTH	TEMPRATURE DEGREE C			HUMADITY %			RAINFALL MM	DAYS
	MEAN MAX	MEAN MIN	AVERAGE	MAX	MIN	AVERAGE		
NOV.'90	28.6	10.6	19.60	86	30	58.00	0.5	1
DEC.'90	24.5	6.1	15.30	91	40	65.50	9.0	1
JAN.'91	22.2	4.5	13.35	90	48	69.00	2.8	1
FEB.'91	26.4	9.9	18.15	89	35	62.00	17.7	3
MAR.'91	33.1	14.0	23.55	79	24	51.50	1.7	1
APR.'91	38.1	19.3	28.70	54	19	36.50	9.0	1
MAY.'91	43.5	24.5	34.00	37	10	23.50	0.2	1
JUN.'91	41.7	27.3	34.50	56	28	42.00	12.4	1
JUL.'91	36.2	26.2	31.20	75.8	50	62.90	460.3	12
AUG.'91	30.1	23.7	26.90	89	69	79.00	428.6	13
SEP.'91	33.4	21.5	27.45	84	46	65.00	2.8	1
OCT.'91	33.5	13.4	23.45	87	29	58.00	-	-
							945	36

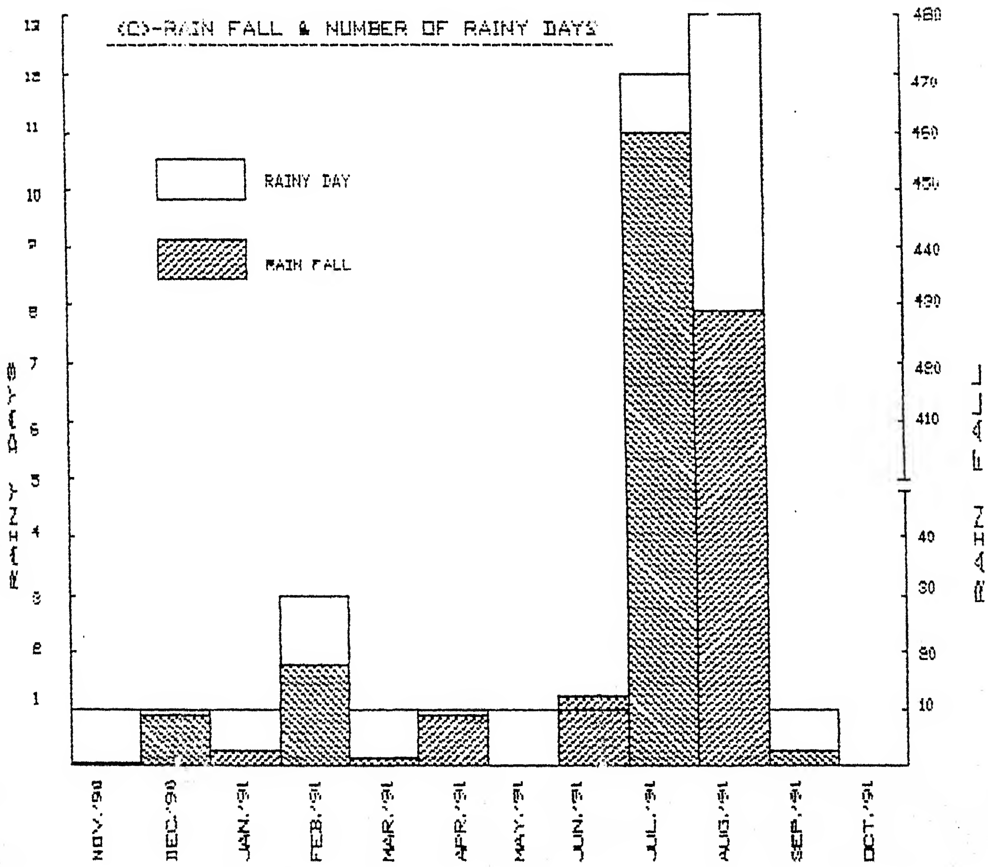

CLIMATIC CONDITION OF JHANSI DURING NOV. '90'-OCT. '91'
(A)-MONTHLY TEMPERATURE VARIATION
MEAN MAXIMUM
MEAN MINIMUM
TEMPERATURE C0
45
40
35
30
25
20
15
10
05
NOV.'90
DEC.'90
JAN.'91
FEB.'91
MAR.'91
APR.'91
MAY.'91
JUN '91
JUL.'91
AUG.'91
SEP.'91
OCT.'91
MONTHS

(FIGURE -1 'A')


CLIMATIC CONDITION OF JHANSI DURING NOV. '90'-OCT. '91'
(B)-MONTHLY HUMIDITY VARIATION
MEAN MAXIMUM
MEAN MINIMUM
HUMIDITY PERCENTAGE
100
90
80
70
60
50
40
30
20
10
NOV,'90
DEC,'90
JAN,'91
FEB,'91
MAR,'91
APR,'91
MAY,'91
JUN,'91
JUL,'91
AUG,'91
SEP,'91
OCT,'91
MONTH'S

(FIGURE-1'B')

CLIMATIC CONDITION OF JHANSI DURING NOV.'90' - OCT.'91'



(FIGURE-1 (C))

temperature varying from 6.0 degree C (January) to 26.5 degree C (June) and mean maximum from 22.9 degree C (January) to 42.3 degree C (May) Table II.

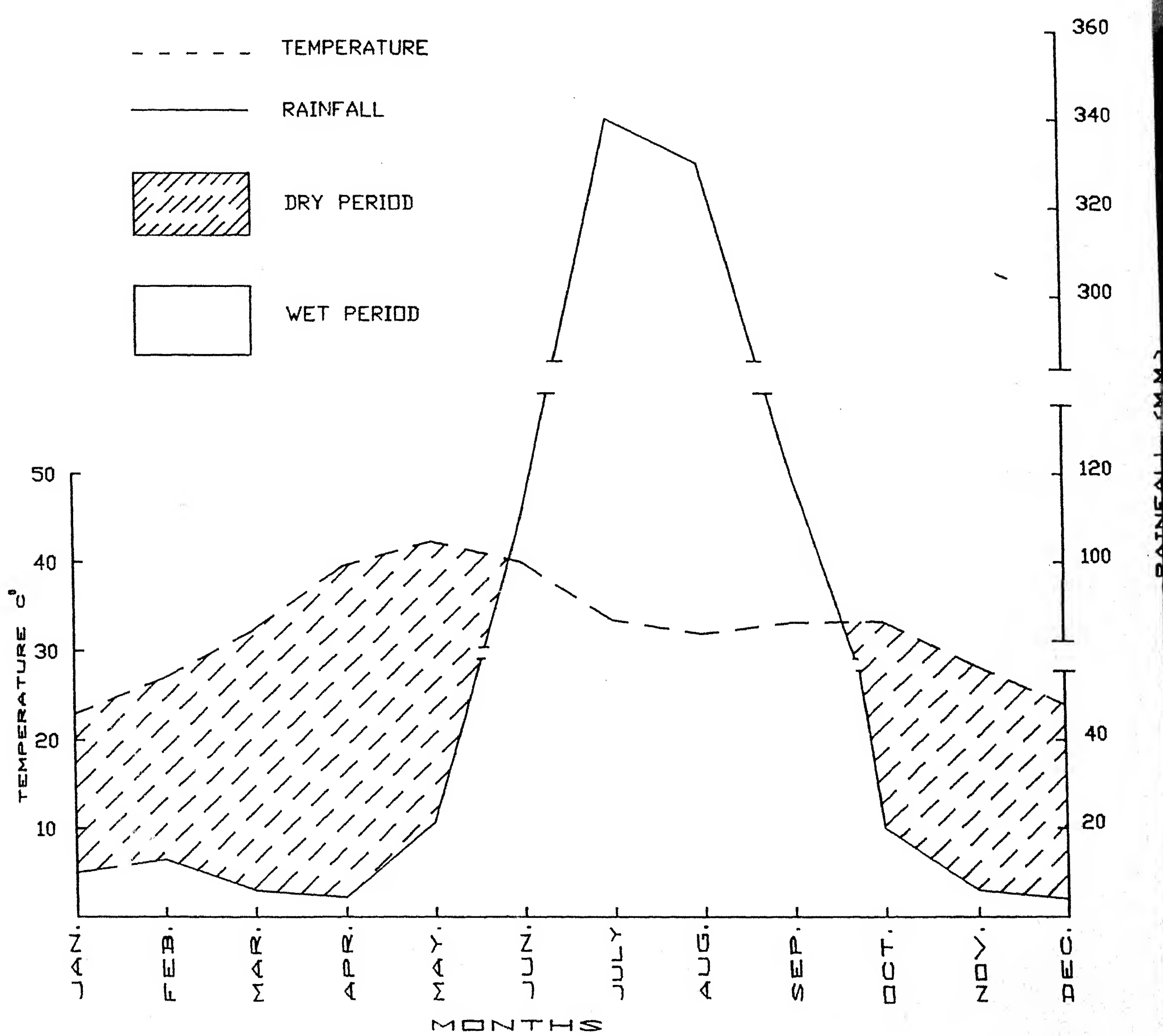
The rainy season is from June to September, July being the wettest period of the year. Almost seventyfive percent of the total average annual rainfall normally occurs during this period figure .I c.

Due to this uneven distribution of rainfall, there is often the defficency of moisture in the soil and atmosphere during summer and winter and thus there is heavy drainage and errosion during rains.

The Winter season starts from mid October and ends in mid February. This season is characterised by low temperature. During this period of study the mean minimum temperature varied from 4.5 degree C (January 1991) to 13.4 degree C (October 1991) and the mean maximum temperature varied from 22.2 degree C (January 1991) to 33.5 degree C (October 1991). December and January were the coldest month of the year.

The Summer season begins from mid February and ends in mid June. This season is characterised by high temperature. During this period of study the mean minimum temperature varied from 14.0 degree C (March 1991) to 27.3 degree C (June 1991) and mean maximum from 33.1 degree C (March 1991) to 43.5 degree C (May

CLIMATIC CONDITIONS OF JHANSI BASED ON DATA OF 15 YEARS



(FIGURE -2)

TABLE - II

CLIMATIC CONDITIONS OF JHANSI BASED ON DATA OF 15 YEARS

MONTH	TEMPRATURE DEGREE C			HUMADITY %			RAINFALL MM
	MEAN MIN	MEAN MAX	AVERAGE	I	II	AVERAGE	
JAN.	6.00	22.90	14.45	84.00	48.00	66.00	9.90
FEB.	9.00	26.90	17.95	81.00	41.00	61.00	12.70
MAR.	12.70	32.30	22.50	67.00	32.00	49.50	3.00
APR.	20.70	39.50	30.10	51.00	27.00	39.00	2.20
MAY.	25.90	42.30	34.10	44.00	25.00	34.50	10.60
JUN.	26.50	40.00	33.25	61.00	40.00	50.50	113.10
JUL.	25.30	33.50	29.40	80.00	66.00	73.00	338.70
AUG.	24.20	31.90	28.05	86.00	69.00	77.50	299.90
SEP.	22.90	33.20	28.05	79.00	58.00	68.00	121.50
OCT.	17.40	33.30	25.35	77.00	42.00	59.50	20.00
NOV.	10.50	28.30	19.40	82.00	36.00	59.00	5.70
DEC.	6.50	24.00	15.25	82.00	46.00	64.00	3.60

1991) . This season remained dry except for occasional rains. May was the hottest month of the season. This season was also characterised by frequent dust, hot winds and storms. Jhansi is one of the hottest place in Uttar Pradesh, where days are hot while nights are cool.

In table II the climatic data of Jhansi for the last 15 yeares have been recorded. A comparision of the climate during the study period with that of proceeding 15 years indicates that rainfall during the year 1990-91 was slightly more (945.0 mm) as compared to the proceeding 15 years average of 940.9 mm.

The effectiveness of the climatic factors like temperature precipitation and length of the dry period can be understood in better way by means of ombrothermic diagram (Fig-2). In this the thermic curves (Mean monthly values of temperature and mean rainfall values) are drawn together. In order to bring out the length of dry period on the graph, the scale of rainfall is taken as double to that of the temperature. The month is considered dry when its mean rainfall is less than twice its mean temperature (T):$P<2T$. A fig 2 indicates that dry period is spread over seven months and only five months (June to Oct.) are considered wet months. Winds acquires maximum speed during summer and rainy season, while it was gentle in mid winter.

SECTION II

MICROBIAL DYNAMISM

CHAPTER - 3

GENERAL INTRODUCTION.

The U.P.State spinning mill is situated on Jhansi Gwalior Road adjoining the air field. The mill apart from having two working units have residential plots for the workers and executives both. About 5000 workers are involved in the day and night shifts. They work in different chambers of the functional unit in which temperature and humidity is maintained according to the requirement to spun cotton into thread.

The bales of cotton arriving at the mill must be inhabiting some micro organism. After the arrival of cotton these are handled by a number of workers. During processing and handling, the cotton fibres might get sprayed with some additional pathogenic and non pathogenic organisms. The organisms now present on the fibres may multiply or remain viable as the temperature and humidity within the mill is quite suitable. The small fibres or lint floating in the air are being constantly trapped in underground tunnels by suction, but still quite a good amount of fibres floats in the air. These fibres may act as vectors for passing the organism from one place to another.

The organism during such colonzation are important as they might be involved to some extent in deterioration of cotton and to some extent in spreading

infection to the workers. Around the working units of the mill some cotton floats in the atmosphere and finally settles on the ground, telephone poles or electric wires, fences etc. These builds up their own ecological niche and host, a number of microbes both in the atmosphere and soil. The organisms sprayed during handling within the mill add to the pollution of the atmosphere. Some toxigenic fungi on cotton have already been reported to occure by Diener et.al., (1976); Nigam et. al., (1960); Bagga (1970) & Lgreere (1973).

There are many reports on the capacity of micro organism to utilise cellulose invitro, Garrett (1962, 1963a, 1966); Hogg (1966); Siu (1991); Rai (1969); Fergus (1969); Reese and Lavinson (1952);Rai (1970); Dwivedi and Singh (1974) that the successfull saprophytic colonization of fungus largely depends upon its cellulolytic ability. This is reflected by the amount of cellulose utilised by the organism. The ability to utilize cellulose is often regarded as an essential feature for saprophytic fungi. In most cases fungi have been studied in pure culture. Garrett (1962) have however, investigated the decomposition of cellulose in soil. Rai (1969) concluded that the successful colonization of fungi may be related to their celluloytic ability. Macer (1961) demonstrated

that the fungus with lowest rate of cellulose decomposition has also shown the longest period of saprophytic survival in buried infected wheet straw. Competitive saprophytic colonization is the invasion of the fibres by the organism in competition with other saprophytic organisms Garrett (1962) defined this as the Colonization of dead matters. Garrett (1963) suggested that in colonization of dead organic matter, the competitive saprophytic ability is one of the main factor, others are inoculum potential and environmental conditions. Garrett (1960) had earlier suggested three factors that are likely to influence the former attribute, (1) intrinsic growth rate of fungus and rapid germination of spores (2) good enzyme production (3) tolerance of antibiotic produce by other microorganism .Competitive saprophytic colonization is obviously one of the normal forms of colonization (The other being parasite) Garrett (1956).

Accordingly in the present investigations periodic isolation of microflora from cotton and air from different sites of the mill, their qualitative and quantitative analysis, their comparative cellulolytic ability and their competitive saprophytic colonization were studied, for the sake of convenience the section has been divided into three subsections.

Subsection A:-Quantitative and qualitative dyanmism of micro organism

This deals with the collection of cotton samples from various sites at an interval of 15 days for one year, From these samples microorganism were isolated and analysed both qualititatively and quantitatively, so as to find out the microbial community, their percentage frequency, absolute number, during various season of the year. Micro-organism were also isolated in addition to these, from air inside and outside the mill to stuty the aerial microflora.

Sub Section - B - Comparative celluloytic ability.

This subsection deals with the studies on comparative cellulolytic ability of the orgaisms.

Only fungi that were frequently found during isolation or fungi with special significance were selected for the present investigations. The method employed for this study was that of Garrett (1962).

Section -C- Competitive saprophytic colonization.

This section deals with the competitive saprophytic colonization of the microflora selected in sub suction B. The method employed for this study was that of Wastie (1961).

SECTION II

SUB SECTION A

QUANTITATIVE AND QUALITATIVE STUDIES

CHAPTER - 4

INTRODUCTION.

The U.P.State spinning mill of Jhansi involves about 5000 workers. Apart from the working unit it consist of residential plots for its employes where the workers live with their families. Thus the total population which is involved with in this mill is about 2000-25000. Workers works in different chambers of the functional unit in which temperature & humidity is maintained according to the requirement to spun cotton into threads.

From the time bales of cotton arriving at the mill it is handled by a number of workers till it is spun into threads. During processsing the cotton fibre might get sprayed with pathogenic or non-pathogenic organisms from the workers & its surroundings.These may multiply or remain viable as the temperature & humidity with in the mill is quite suitable. The small fibres floating in the air are being constantly trapped in chambers by suction but quite a good amount of fibres float in the air. These fibres may act as vectors for passing the organism from one place to another. The organism during such colonization are important as they might be involved in deterioration or causing infection to the workers. Some toxigenic fungi on cotton have been reported to occur by Diener et. al., (1976); Nigam (1960); Marion et. al., (1969); Bagga (1970).

There are many reports an the capacity of micro-organism to utilise cellulose <u>in vitro</u> Garrett (1962,1963); Hogg (1966); Reese & Lavison (1952); Rai(1970); Dwivedi & Singh (1974).

Nigam (1960) have studied microbial degradation of cotton cellulose in soil. To understand the microbial dynamism it is necessary to make a quantitative & qulitative study of the microflora involved . Nigam <u>et. al.</u>, (1960); Diener <u>et. al.</u>, (1976), Ahmad & Gulatia (1943); Sharma & Roy (1979); Shukla & Tandon (1960); Simpson <u>et. al.</u>, (1969); Ashworth <u>et. al.</u>, (1971); Simbya <u>et. al.</u>, (1969)isolated number of fungi, bacteria,& actinomycetes from cotton fibre. Bagga (1970) made pathogenic studies of 36 organisms, associated with cotton boll. **<u>Aspergillus flavus, A. fumigatus, A. nidulans, A. niger A. ustus, Cladosporium herbarum, Fusarium moniliforme, F.Solani, Rhizopus stoniger, Trihoderma viridi, Verticillum</u>** etc.were used by him. Nigam(1960) indentified fungi which appeared on burried cotton fabrics & filter paper in soil, together with above observations on the period of appearence of various group of organisms . He quantitatively obtained bacteria in the range of 2,000,000,actinomycetes 80,000 & fungi 16,000 per gram. Among fungi he found the presence of **<u>A.flavus, A. fumigatus , A. oryzae, A.</u>**

terreus, A. ustus, A. niger, Pencillium sp., Cladosporium Sp., Alternaria sp, Rhizopus nigricans & Tricoderma viridi. Simpson & Marsh(1969) also found Alternaria, A. flavus, A. niger, Fusarium sp., Rhizopus stoniger commonly infecting cotton fibre. They also observed the association of fungus with that of fibre & described the type of binding or rolling of fungal hypae on these fibres. Marsh & Bollenbacher (1949) described fungi concerned in fibre deterioration. Most of these workers studied cotton fibre deterioration mainly at the time of boll opening. Large number of microbes are involved in its degradation. Bacteria & actinomycetes are largely present during the initial stage of degradation and cause considerable decay , fungi appear later & complete the process of degradation, Nigam et. al., (1960). Bacteria in relation to decomposition are usually divided into functional groups (Gyllen Berg & Eklund, 1974).

(a) The indigenous organism, whose number in soil are supposed to remain unaffected by dead organic matter.

(b) The Zygnogenous organism,which are actively involved in the decomposition of dead organic matter.

Although bacteria are widespread in nature but these are small in size & thus probably represent less

than half the microbial protoplasm. Thus in terms of protoplasmic proportions in most environments fungi obviously constitute the primary population where as bacteria appear as secondary population. Among these microbes minority in normal circumstances appear to require the presence of a living host (Alexander 1961). The remaing are either facultative parasites able to live on the dead remains of the host or saprophytes .

During the last 60 years a numbers of techniques have been developed for the isolation & study of fungal flora,Warcup (1960,1967); Parkinson et. al., (1971), Johnson & Curl(1972).each with its own advantages. The approach most frequently used for enumeration is the plate count in which dilutions of the specimen in steril water are plated on a suitable agar medium. Microstatic agents such as penicillin, rose bengal & streptomycin were included in the media to explore, fungal organisms for obvious reasons.

Alexander (1961) postulated that the actinomycetes develop far more slowly than most of bacteria & fungi & are rather ineffective competitors in the early stage of the decomposition. They are more prominent in the later stages of the process. The late apprearence of actinomycetes is attributed to their ability to degrade the more complex organic substances.

From the above account it will the evident that in order to understand the microbial dynamism of cotton fibre it is necessary to make a successive periodic study of the mycoflora involved & there quantitative & qualitative analysis. So as to find out microbial colonization ,their absolute number & percentage frequency during various seasons of the year. To achieve the above objective studies were conducted in the following lines.

(a) <u>Isolation of micro organism & there quantitative & qualitative analysis.</u>

Micro-organism were isolated from cotton fibre collected from 3 different sites (store room, absorbing, tunnel & spinning chambers within the mill & from air or lint floating within the mill & out side the mill. The isolation process was done at interval of 15 day for 1 year. Soil dilution plate method of Waksman (1927) as standarized by Brierley <u>et. al.</u>, (1928) was adopted for isolation of microorganism from cotton fibre. Isolated fungi were analysed both quantitatively & qualitatively while bacterial population was analysed quantitatively only, however few pathogenic forms which come accross were picked up separatly & given special consideration. To study the microbial colonization their percentage frequency & absolute number were recorded at successive isolations.

(b) <u>Results and conclusion</u>- from the qualitative and quantitative datas obtained the analysis was done in this section, in which the various sites were compared for their frequency and absolute number.

Chapter - 5

Experimental

ISOLATION OF MICROFLORA

Cotton samples for isolation of microflora were were collected from the following sites of the spinning mill.

Site No.I:-Absorbing channel-Through out the working unit there is an underground absorbing channel having suction to suck the cotton fibre floating in the air. In this channel thus there is deposition of cotton fibre & dust.

Site No-2 -The store room -There is a large room in which bales of cotton which arrive for spinning are kept.Comparatively here only few workers are involved and cotton is generally kept for a short period of time i.e.for a month or so since it is being used up constantly.

Site No.3- Spinning Chamber - There are two units A & B in the spinning mill each unit have three chambers to spun the cotton . In first chamber bales of cotton are being threshed mechanically with the help of automatic threshers , in second unit cotton rolls are being prepared which finally goes into third unit where fine threads are drawn out of these rolls & 3 -4 threads are then spun a into single thread & these are than rolled into reals. There is a separate packing room where the

reels are packed for marketing.

Large number of workers are involved in these chambers & rooms. Cotton fibres , lint & dust float in the air, density of which is highest in first chamber & gradualy decrease in the third. These fibres, lint & cotton have the microorganism of their own together with some which are incorporated during handling. During handling some pathogenic organisms also get involved from the workers who act as carriers for various organisms.

The temperature of these chambers are maintained at about 34 degree - 36 degree centigrade through out the year . Some water is constantly sprayed to get the desired humidity. Cotton samples were collected from the floor of the 6 chambers & mixed togather & was used as a composite sample for site No.3.

Site No.4- Microorganism of the atmosphere inside spinning chambers - Microorganism from the air inside the spinning chambers were isolated directly on exposed petriplates having the desired poured & solidified medium. Petriplates were exposed in each of the 6 chambers & average number of organisms developed were noted & tabulated in the table.

Site No.5 Micro organism of the atmosphere outside the spinning mill - For isolating micro-organism

outside the mill , the petriplates with desired, poured & solid media were exposed outside working unit of the spinning mill, residential quarters of the workers & residential quarter of the official staff . Average No. of organism developed were incorporated in the table.

PROCEDURE

The isolation process was done at an interval of 15 days for 1 year. Cotton samples were collected from the floor of absorbing charnel, spinning chamber & from store room at each sampling. Samples were collected by means of sterlized forceps and brought to the laboratory in separate sterlized petridishes.

Microorganism from air inside & out side the mill were isolated on petriplates having the desired medium.

The soil dilution plate method of (Wakman, 1927) as standardized by Brierley et. al., (1928), was adopted for the isolation of microflora from collected samples. Samples from each sites were brought to the laboratory in sterile petriplates & were added to 250ml sterile round bottom flasks containing 100ml of sterilized water. The flasks were thoroughly shaken in a mechanical wrist action shaker for half an hour & were used to prepare desired dilutions.

(i) The fungal isolation were made by preparing 1:1000 dilution of the above sample & plated in Peptone Dextrose Agar with Rose bengal & Streptomycin (Martin, 1950 ; Johnson1957).

The plates were incubated at 32 $\pm$ 2 degree centigrade and observed after 4-7 days.

(ii) For isolating bacteria 1:10,000 dilutions were prepared & plated in blood Agar,and soil extract agar medium & incubated at 32 degree centigrade for 2-5 days.

Since most of the time the temperature inside the mill remained near about 34 degree C, so the temperature of incubation for both bacteria & fungi was kept at 32 $\pm$ 2 degree centigrade during isolation process. The incubation temperature was kept in the proximity of temperature which was maintained inside the working units. This was done so that the organism existing at the temperature inside the mill could develop in the incubation plates.

The bacterial poulation was analysed quantitatively where as the fungal population was analysed both quantitatively & qualitatively . However a few bacteria which were of pathogenic nature were picked up separately & kept for further study. The data obtained are tabulated for the percentage occurence, absolute number & percentage frequency at 15 days

intervals for the entire experimental period of one year. Counting was done using colony counters.

Identification were done on isolation plates as far as possible & transferred to potato dextrose agar or nutrient agar for further indentification & culture maintenance for further use. The suspension was filtered on previously weight filter paper & dried at 80 degree centigrade till constant weight, for obtaining the final dry weight of the cotton. From this the number of organisms per gram dry weight of cotton was calculated.

For microorganism floating in the air, the previously poured petriplates were exposed in the atmosphere both within the mill & outside the mill. These plates with desired media were generally exposed for 1-2 minutes except for the isolation of <u>Mycobacterium</u> <u>tuberclosis</u> where the exposure was to be prolonged for about 20 minutes on Lowenstien Jensen Media.

For Fungi Peptone dextrose agar with rose bengal & Streptomycin (Martien 1950 , Johnson, 1957) media, were used & for bacteria, soil extract, blood Agar media & Lowenstien Jensen Media were used.

The frequency percentage for different fungi were calculated using Raunkicaers (1934) formula, as follows.

% Frequency =

$$\frac{\text{No.of sampling units in which the species occured}}{\text{Total number of units studied.}} \times 100$$

Results for above analysis were given in table III to XII & fig. 3 to 12.

CHAPTER - 6

RESULTS & CONCLUSIONS

Quantitative & Qualitative analysis.

In this study the quantitative & qualitative assessment of the microflora isolated from cotton fibre and air , in & around the spinning mill was done at an intervel of 15 days upto 1 year. The bacterial population was analysed quantitatively where as the fungal population was analysed both quantitatively & qualitatively. However during isolation the author came across a few pathogenic forms of bacteria. These were carefully picked up for further studies. For convenience five differnt sites as discribed above were decided & the isolating samples were picked from the same site on every occassion.

Site No.1-Absorbing channel :-

Quantitative analysis-

absolute Number of fungi & bacteria isolated from site I of spinning mill was determined by Waksman's dilution method' & is expressed in term of number per gram dry weight of cotton in table III fig. 3.

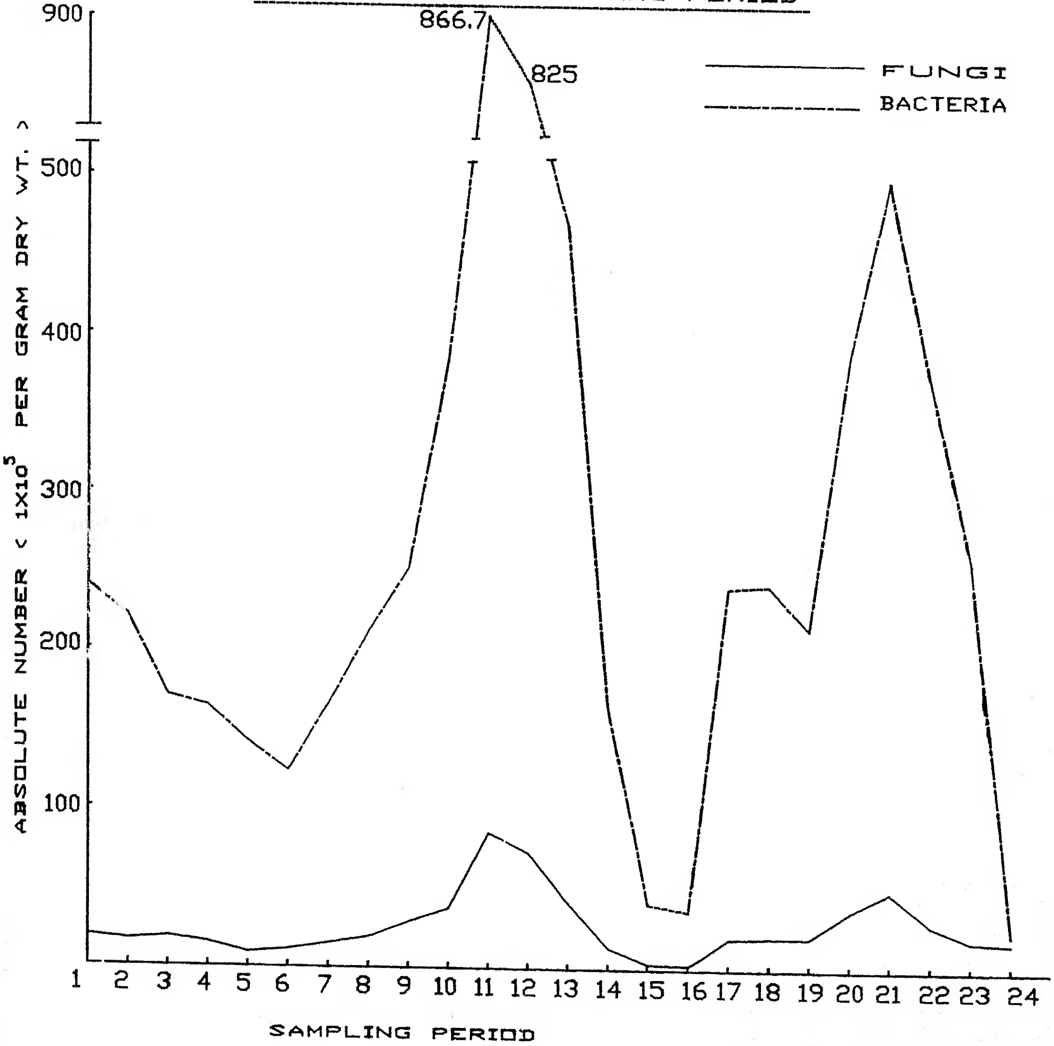
A perusal of the data reveals that the absolute number of fungi remained almost the same up to IV Sampling period, than declined on the V sampling, thereafter gradually increased & came to a miximum at the XI sampling period. This gradually declined up to

T A B L E - I I I

Absolute number of Fungi and bacteria in 'Site - I' at differnet sampling periods (expressed as 1×10^{5} per gram dry weight of cotton).

Sampling period	Absolute No. of fungi	Absolute No. of bacteria.
I	18.66	241.8
II	16.19	221.9
III	18.19	171.7
IV	15.05	165.3
V	8.85	143.0
VI	11.05	124.6
VII	15.00	167.1
VIII	19.18	213.2
IX	28.85	252.8
X	37.40	385.6
XI	85.5	866.7
XII	72.59	825.0
XIII	41.59	470.7
XIV	13.66	165.6
XV	3.64	42.2
XVI	2.89	37.5
XVII	19.49	241.8
XVIII	20.42	243.6
XIX	20.83	216.0
XX	37.40	389.1
XXI	49.86	500.0
XXII	29.09	375.0
XXIII	19.09	260.7
XXIV	17.91	240.4

ABSOLUTE NUMBER FUNGI & BACTERIA IN 'SITE -I' AT DIFFERENT SAMPLING PERIOD



(FIGURE-3)

XVI sampling following again a gradual rise upto XXI sampling period . This again declined up to the last period of isolation i.e. XXIV.

The maximum Number of fungi were recorded in eleventh sampling i.e.85.5x10^5 /gm dry wt. of cotton and minimum Number fungi were recorded in the XVI sampling i.e.2.89x10^5 per gram dry weight of cotton.

The monthly analysis reveals that absolute Number of fungi were in creased during March & April reaching to a maximum level in April and then during May - June absolute number of fungi were decreased & the minimum Number of fungi were recorded during June,this might be the effect of very high atmospheric temperature during this month i.e.42 degree centigrade during day time, when samples were picked up. Fungal population again increased during July, August & September. During October, November, December, January & February when atmospheric temperature was low absolute number of fungi decreased as compared to that of August & September.

The bacterial absolute number followed almost the same trend. Table III and fig : 3. Shows that the absolute number from the initial sampling declined up to the VI sampling period. There after increased & reached the maximum level in the XI sampling period, this number decline up to XVI sampling stage &

considerably increased up to XXI stage & then followed a decline up to the last sampling i.e.XXIV. Maximum number of bacteria were recorded in XI sampling i.e. 866.75×10^{5} per gram dry wt. of cotton & minimum number. of bacteria were recorded in XVIth sampling i.e. 37.5×10^{5} per gram dry wt. of cotton.

Qualitative analysis :-

In all twenty two species belonging to 13 genera were found colonizing the cotton fibre from'site I', the percentage colonization & frequency of fungi are given in table -IV & fig - 4.

Aspergillus niger, *Aspergillus flavus*, *Penicilium citrinum* & *Rhizopus nigricans* Showed abundant colonization trough out the sampling. *Aspergillus niger* & *Aspergillus flavus* were found to be most dominating form and occurred during every sampling. *Aspergillus sulphureus* occured on 10 occassion, *Aspergillus fumigatus* on 9, *Aspergillus terreus* & *Fusarium oxysorum* & *Phycomyces sp* on 8, *Aspergillus oryzae* on 7 & *Aspergillus nidulans*, *Alternaria alternata* & *Tricoderma viridi* occurred on 6 occassion respectively.

Chaetomimum globosum, *Cladosporum sp.* appeared only twice. While *Paecilomyces varioti*, *Virticillum*

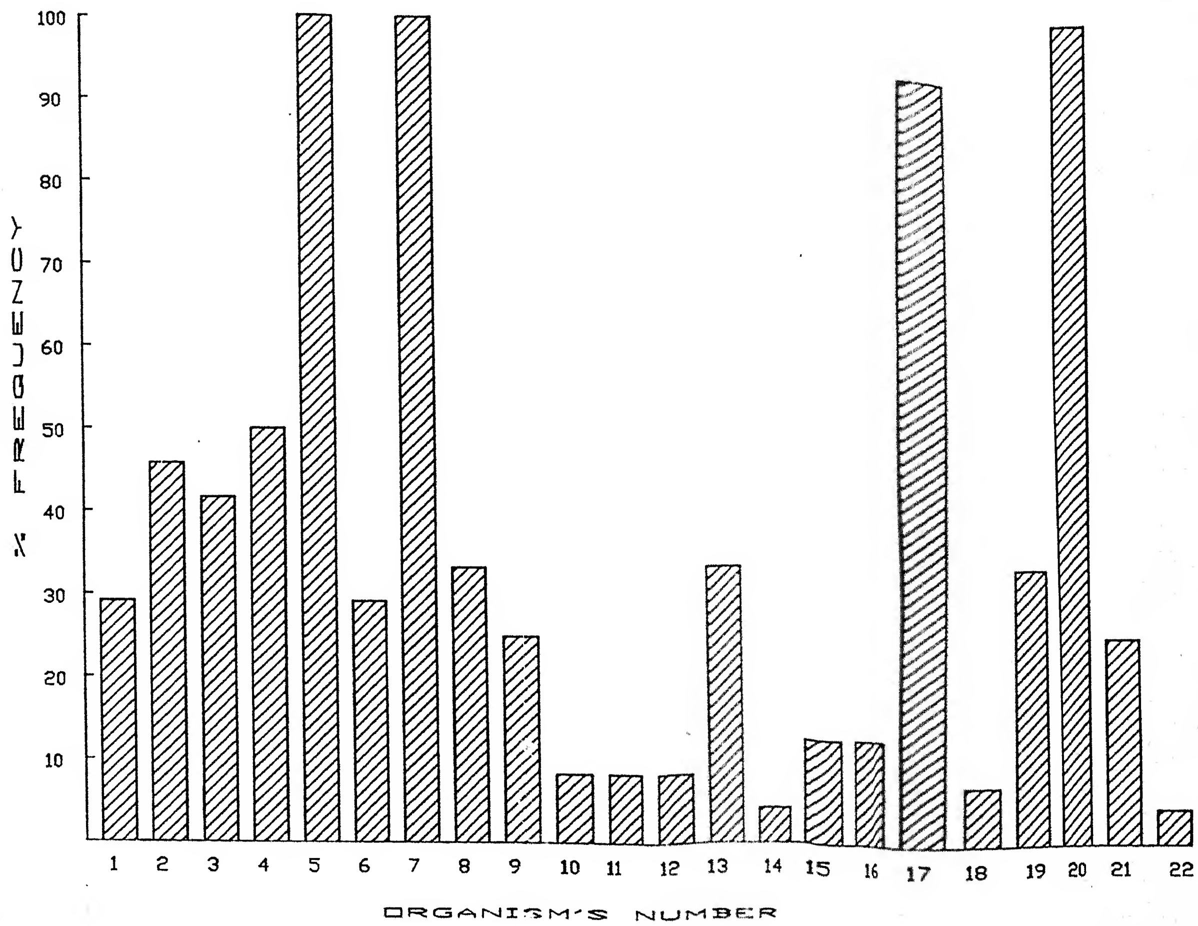
TABLE -IV

PERCENTAGE COLONIZATION & FREQUNCY OF DIFFERENT FUNGI 'SITE 1'AT DIFFERENT SAMPLING PERIODS

I) SAMPLING PERIOD AT INTERVEL OF 15 DAYS
II) INCUBATION TEMPERATURE 32 ± 2°C

S.No.	ORGANISMS	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	FREQUENCY
1	Alternaria alternata	-	-	-	-	-	-	-	5	-	-	-	3.5	5.7	6.2	-	-	-	-	4	-	-	2.7	-	-	29.2%
2	Aspergillus sulphureus	9.09	8.6	-	-	-	-	-	-	5.8	--	-	7.1	5.7	-	-	15.7	-	8.6	-	-	4.3	-	12.5	4.3	45.8%
3	Aspergillus fumigatus	-	4.4	-	10	-	-	5	-	-	6.4	5.8	-	-	-	8	-	-	-	-	-	2.1	5.5	-	4.3	47.1%
4	Aspergillus ustus	-	-	-	5	9.09	-	-	5	-	3.1	-	10.7	2.8	18.7	-	15.7	4.5	-	8	6.4	6,5	5.5	-	-	50%
5	Aspergillus niger	54.5	39.1	61.5	35	45.4	44.4	40	35	35.2	35.4	41.1	28.5	34.2	37.5	32	26.3	10.9	34.7	24	38.7	39.1	30.5	33.3	47.8	100%
6	Aspergillus oryzae	-	-	-	10	-	-	-	5	-	-	2.9	-	8.5	-	-	-	-	-	-	-	5.5	5.5	-	9.3	29.2%
7	Aspergillus flavus	18.18	21.7	30.7	20	27.2	11.2	25	27.5	23.5	22.5	32.3	21.4	22.8	25	36	31.5	22.7	26	20	25.8	17.3	19.4	20	17.3	100%
8	Aspergillus terreus	-	4.3	-	-	-	5.5	-	10	-	9.6	-	-	-	-	-	5.2	-	-	8	-	-	11.1	4.1	-	33.3%
9	Aspergillus nidulans	-	-	-	-	-	-	10	-	-	-	-	7.1	-	10	-	-	-	-	-	6.4	4.3	-	8.3	4.3	25%
10	Basidiobolus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	2.1	-	-	-	8.3%
11	Chaetomium globosum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.3	-	-	-	8.3%
12	Cladosporium sp.	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	3.2	-	-	-	-	8.3%
13	Fuasrium oxysporum	-	-	-	5	-	-	-	-	5.00	-	5.8	-	-	-	-	-	-	4.3	4	-	2.1	2.7	4.1	-	33.3%
14	Fusarium solani	-	-	-	-	-	-	-	-	-	3.2	-		-	-	-	-	-	-	-	-	-	-	-	-	4.2%
15	Mucor hydrophilus	-	-	-	-	-	-	-	-	3.00	-	-	-	-	-	-	-	-	-	-	3.2	-	2.7	-	-	12.5%
16	Paecilomyces varioti	-	-	-	-	-	-	-	-	2.9	-	-	-	-	-	-	-	-	4.3	-	-	2.1	-	-	-	12.5%
17	Penicillium citrinum	9.04	3.0	-	10	-	11.1	10	9	8.8	6.4	5.8	19.2	5.7	6.2	8	10.5	4.5	8.6	16	6.4	8.6	8.3	8.3	8.6	91.7%
18	Penicillium sp.	-	-	-	-	-	5.5	-	5	-	-	-	3.5	-	-	4	-	-	-	-	-	-	-	-	-	7.0%
19	Phycomyces sp.	-	-	-	-	-	5.5	-	-	2.9	-	-	-	2.8	-	4	-	-	4.3	4	6.4	-	-	4.1	-	33.3%
20	Rhizopus nigricans	4.09	8.6	7.6	5.6	18.1	5.5	5	5	5.8	9.6	2.9	7.1	2.8	6.2	4	-	9	4.3	8	3.2	4.3	5.5	4.1	8.6	95.8%
21	Trichoderma viridi	-	-	-	-	-	-	5	5	2.9	3.2	-	-	-	-	-	-	-	-	4	-	2.1	-	-	-	25%
22	Verticillium alboatrum	-	-	-	-	-	-	-	-	5	-	-		-	-	-	-	-	-	-	-	-	-	-	-	4.2%

PERCENTAGE FREQUENCY OF DIFFERENT FUNGI AT 'SITE-I'



(FIGURE -4)

alboatrum, Basidibolus sp. Fusarium solani, Mucor hydrohilus only once.

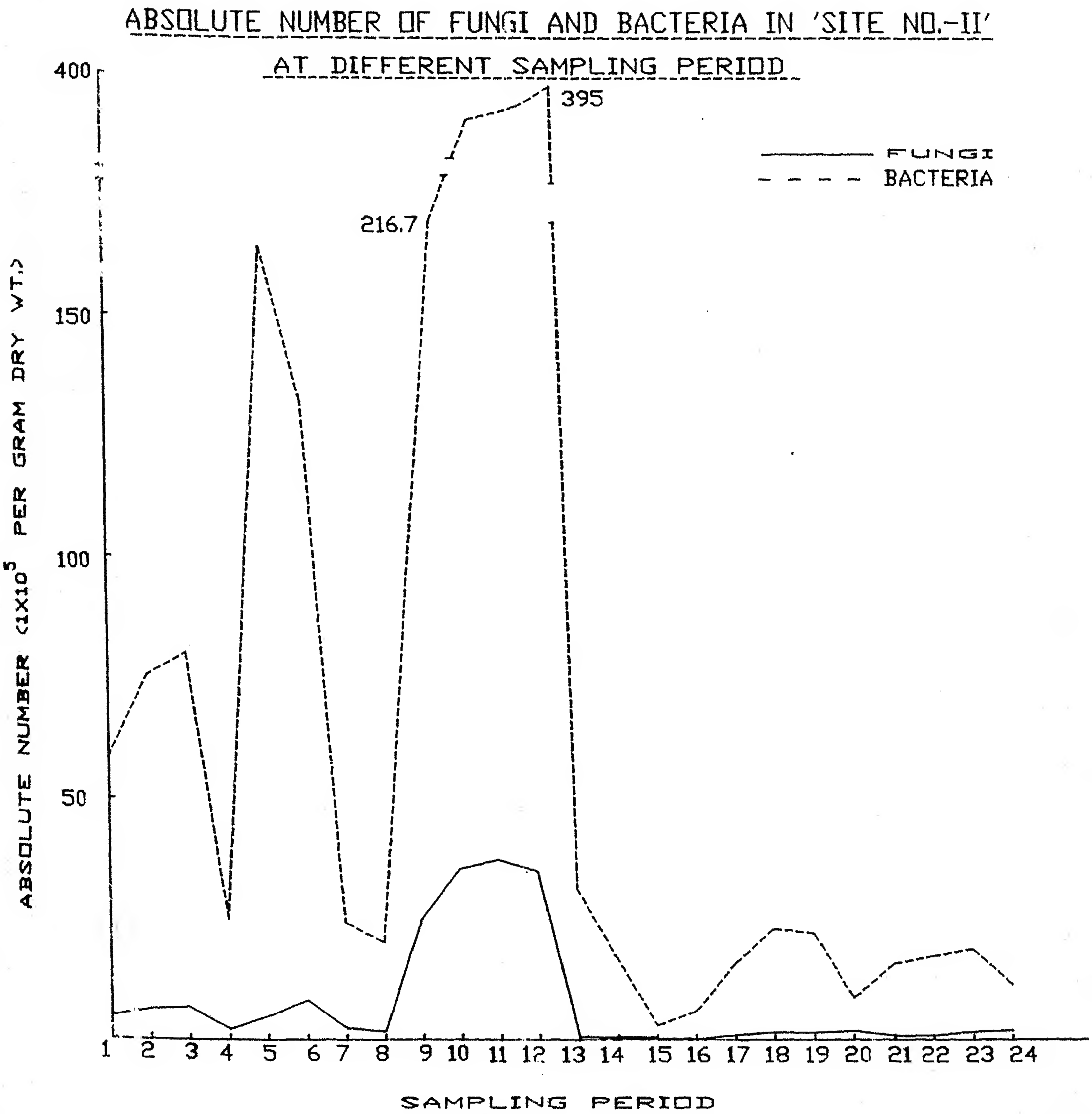
Aspergillus niger & Aspergillus flavus had 100% frequency followed by Rhizopus nigricans & Penicilium citrinum which showed 95.8% & 91.7% frequency respectively, Aspergillus ustus showed 50% frequency. The percentage frequency of Aspergillus sulphureus, Aspergillus fumigatus, Aspergillus terreus. Phycomyces sp. Trichoderma viridi, Fusarium oxysporum, Aspergillus oryzae & Alternaria alternata varied between 25% to 45%.

Basidiobolus sp. Chaetomium globosum, Cladosporum sp. Penicillium sp. Paecilomyces varioti, Mucor hydrophilus had low percentage frequency i.e. between 8% to 13% while Verticillium alboatrum & Fusarium solani had the lowest percentage frequency i.e. 4.2%. Percentage frequncy of different fungi are shown in the fig. 4.

Site-II-The store room- (room where bales of cotton are kept)

(1) Quantitative analysis-

The absolute Number of fungal & bacterial species isolated from "site II" at different sampling periods are shown in table V fig 5 .


ABSOLUTE NUMBER OF FUNGI AND BACTERIA IN 'SITE NO.-II'
AT DIFFERENT SAMPLING PERIOD
400
150
100
50
395
216.7
FUNGI
BACTERIA
ABSOLUTE NUMBER (1X10^5 PER GRAM DRY WT.)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
SAMPLING PERIOD

(FIGURE-5)

T A B L E - V

Absolute number of Fungi and bacteria in 'Site - II' at differnet sampling periods (expressed as $1x10^5$ per gram dry weight of cotton).

Sampling period	Absolute No. of fungi	Absolute No. of bacteria.
I	4.82	58.9
II	6.25	76.0
III	6.96	80.6
IV	2.19	25.6
V	4.86	165.0
VI	8.23	132.9
VII	2.43	24.8
VIII	1.72	20.7
IX	25.0	216.7
X	35.8	368.5
XI	37.5	385.0
XII	35.0	395.0
XIII	0.65	31.8
XIV	0.54	17.5
XV	0.29	03.6
XVI	0.18	06.5
XVII	0.99	16.5
XVIII	1.55	23.6
XIX	1.45	22.6
XX	1.85	09.2
XXI	0.82	16.4
XXII	0.92	18.0
XXIII	1.66	12.1
XXIV	2.05	12.1

From Ist to IVth sampling stages the absolute number of fungi remained more or less the same, than increased in IVth & Vth sampling followed by decline up to VIIIth sampling. The absolute number of fungi increased during IXth to X Ith sampling period & came to a maximum at the XIth sampling XIIIth sampling there was a sharpfall in the absolute number of fungi followed again by a slight rise from XVIIIth sampling but the absolute number of fungi remained low with little fluctuation. In XXI & XXII sampling again absolute number of fungi decreased followed again by a gradual rise up to last sampling i.e.XXIVth .

The maximum number of fungi were recorded in XIth sampling period i.e. 37.5 X 10^5 per gram dry weight of cotton & minimum No of fungi were recorded in XVIth sampling i.e. 0.18 X 10^5 per gram dry. wt. of cotton.

The monthly analysis reveals that effect of atmospheric temperature on absolute number of fungi was same as noted while studying the number of fungi of site 'I' i.e. when atmospheric temperature was very high i.e.42 degree centigrade (during day when samples were up picked up), the number of fungi decreased when atmospheric temperature was low in November, December, January & February & also less number of fungi were recorded as compare to the samples worked out during March & April when the absolute number of fungi reached

to a maximum level.

Similar trend was followed by bacteria i.e.the absolute number of bacteria remain more or less same in Ist -IIIrd sampling. Number of bacteria increased in Vth sampling followed by gradual decline up to VIIIth sampling. Then again a gradual rise up to XIIth sampling & comming to a maximum at the XIIth sampling. Again absolute No. bacteria decreased from XIIIth. to XVIth. sampling, followed by slight rise in number in XVIIIth & XIXth sampling. From XXIst to last XXIVth sampling number of bacteria remain almost the same with little fluctuation. Mxaimum number of bacteria were recorded in XIIth sampling that is 395.0×10^{5} /gram dry weight of cotten & minimum number of bacteria were recorded in XVth sampling i.e. 3.6×10^{5} /gram dry weight of cotten.

<u>Qualitatve analysis-</u>

The percentage colonization of different fungi at 'site II' at different sampling period were recorded in table VI & fig - 6.

Eighteen species belong to 11 genera were found colonizing the cotton fibre of site II.

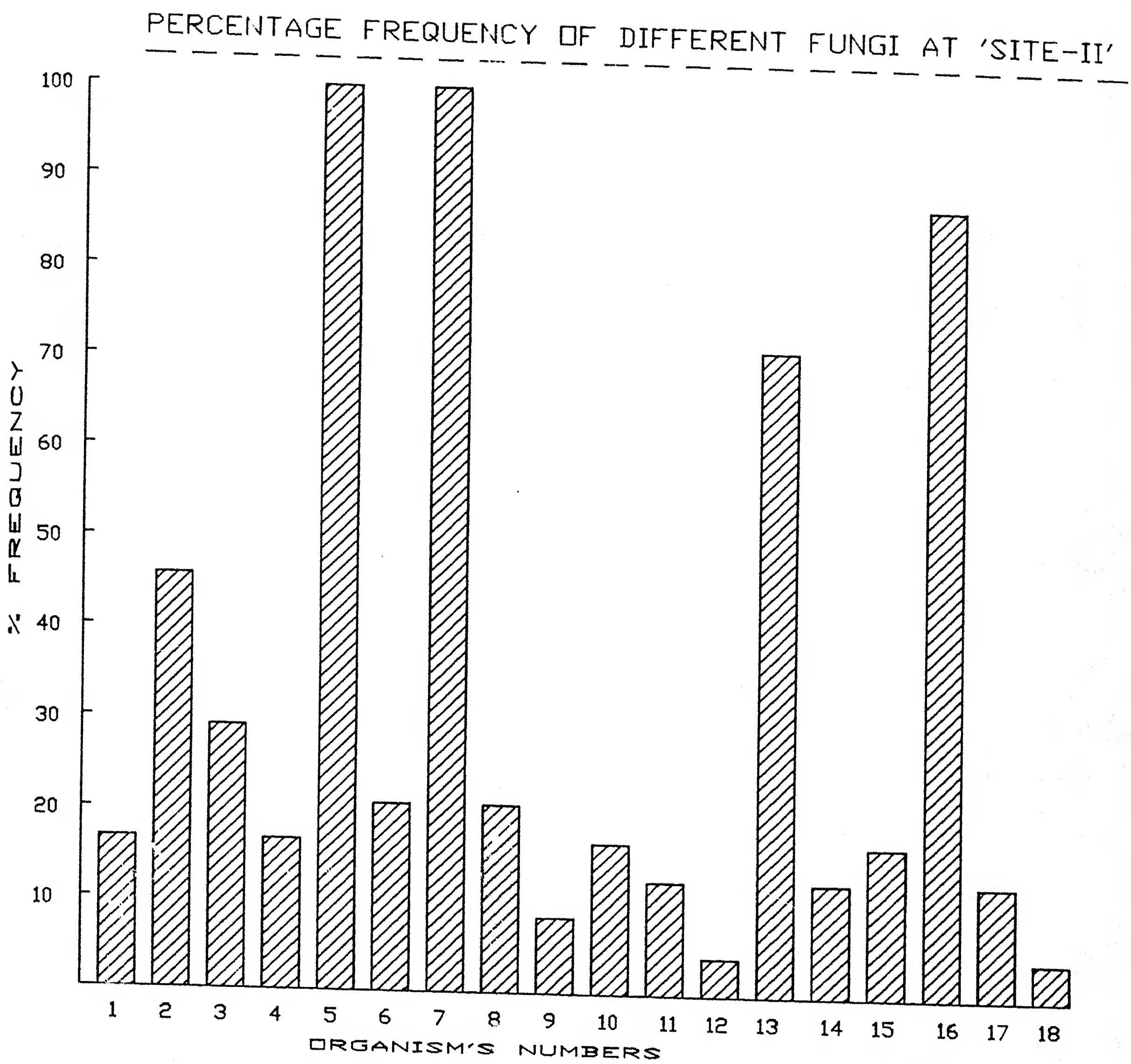
<u>**Aspergillus niger, Aspergillus flavus, Penicillium citrinum & Rhizopus nigricans**</u> showed abundent

TABLE - VI

PERCENTAGE FREQUENCY / COLONIZATION OF VARIOUS FUNGI ' SITE 2'AT DIFFERENT SAMPLING PERIOD

I) SAMPLING PERIOD AT INTERVEL OF 15 DAYS
II) INCUBATION TEMPERATURE 32 ± 2°C

S.No.	Organism	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	FREQUENC
1	Alternaria alternata	-	-	-	-	-	-	5	-	6.6	-	-	7.1	-	-	-	-	-	5.3	-	-	-	-	11.1	-	16.7%
2	Aspergillus sulphureus	3.57	-	18	-	20	20	-	-	-	-	-	7.1	-	-	-	-	8.3	10.5	-	5	11.1	10	-	-	45.8%
3	Aspergillus fumigatus	-	2.8	9	-	-	-	2.5	7.1	-	-	-	-	-	-	-	-	-	5.3	-	-	-	-	5.6	4.5	29.2%
4	Aspergillus ustus	-	-	-	-	-	-	-	7.1	6.6	-	-	-	-	-	-	-	-	-	-	-	-	40	38.9	-	16.7%
5	Aspergillus niger	50	60	36.36	57.14	40	-	37	4.8	33.3	43.6	53.3	28.6	40	50	25	40	50	36.8	31.3	50	33.3	-	11	50	100%
6	Aspergillus oryzae	3.5	-	-	-	-	10	10	-	-	-	-	-	-	-	-	-	8.4	-	12.5	-	-	30	22.2	-	20.8%
7	Aspergillus flavus	32.14	11.4	18.18	28.5	20	20	15	25	23.6	28.5	20	21.4	20	25	12.5	2	16.6	21	25	25	22.2	-	5.5	9.2	100%
8	Aspergillus terreus	-	-	-	-	-	-	2	3.5	-	-	-	-	-	-	-	-	-	-	6.2	-	-	-	-	-	20.8%
9	Basidiobolus sp.	-	-	-	-	-	-	-	-	-	-	-	7.1	-	-	-	-	-	-	-	-	-	10	-	-	8.4%
10	Cladosporium sp.	-	5.7	-	-	-	10	2	-	-	-	-	-	-	-	-	-	-	-	6.2	-	-	-	-	-	16.7%
11	Chaetomium globosum	-	2.8	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5%
12	Mucor hydrophilus	-	-	-	-	-	-	2.5	-	-	-	-	-	-	-	-	-	-	-	6.2	-	-	10	-	-	4.2%
13	Penicillium citrinum	3.57	8.5	9.09	-	20	20	10	3.5	13.3	14.2	6.6	14.3	10	32.5	37.5	40	8.3	10.5	-	10	11	-	-	13.6	71.42
14	Penicillium sp.	-	-	-	-	-	-	2	-	3.3	4.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5%
15	Phycomyces sp.	-	-	-	-	-	-	2	-	3.3	-	6.7	7.2	-	-	-	-	-	5.3	12.5	5	22.3	-	5.6	4.5	16.7%
16	Rhizopus nigricans	7.14	8.5	9.09	14.2	-	-	5	7.1	6.6	4.7	6.7	7.2	30	12.5	25	-	8.3	5.3	-	-	-	-	-	18.2	87.5%
17	Fusarium oxysporum	-	-	-	-	-	-	2.5	-	3.3	-	6.7	-	-	-	-	-	-	-	-	-	-	-	-	-	12.5%
18	Verticillium alboatrum	-	-	-	-	-	-	-	3.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.2%


PERCENTAGE FREQUENCY OF DIFFERENT FUNGI AT 'SITE-II'
% FREQUENCY
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
ORGANISM'S NUMBERS

(FIGURE-6)

colonization throughout the sampling.Aspergillus niger was found in higher percentage colonization, followed by Aspergillus flavus. Aspergillus niger- was found to be most dominating form during every sampling. Mostly the species of Aspergillus dominated i.e. Aspergillus sulpureus, Aspergillus fungigatus, Apergillus oryzae, Aspergillus terreus & Aspergillus ustus were frequently isolated during earlier or latter isolations.

Fusarum oxysporium.Phycomyces sp, cladorsporium sp. Chaetomium globosum, Penicillium sp., Alternaria alternata, appeared in 3-5 isolations. Basidiobolus sp. appeared twice, while Mucor hydrophilus & Verticellum alboatrun appeared only once during VII & VIII isolation respectively.

Aspergillus niger, Aspergillus flavus have 100% frequency, followed by Rhizopus nigricans & Pencillium citrinum which have 87.5% & 71.42% frequency respectively. Aspergillus sulphureus had 45.8% frequency. Alternaria alternata ,Aspergillus fumigatus, Aspergillus ustus, Aspergillus terreus, Chaetomium globosum Phycomyces sp. had percentage frequency between 16% - 30%. Fusarium oxysporum & Cladosporium sp. had 12.5% frequency. while Verticillum alboatrun & Mucor hydrophilus had lowest 4.2% frequency. Percentage frequency are shown in table VI & Fig. 6.

Site III- Spinning Chambers.

Three chambers are situated inside the mill to spun the cotton. Large number of workers are involved in these chamber and significant amount of cotton fiber, cotton lint & dust float in air. During handling some pathogenic organisms also get involved from the workers.

The temperature of these chambers are maintained to 34 degree C - 36 degree C throughout the year.

Quantitative Analysis.

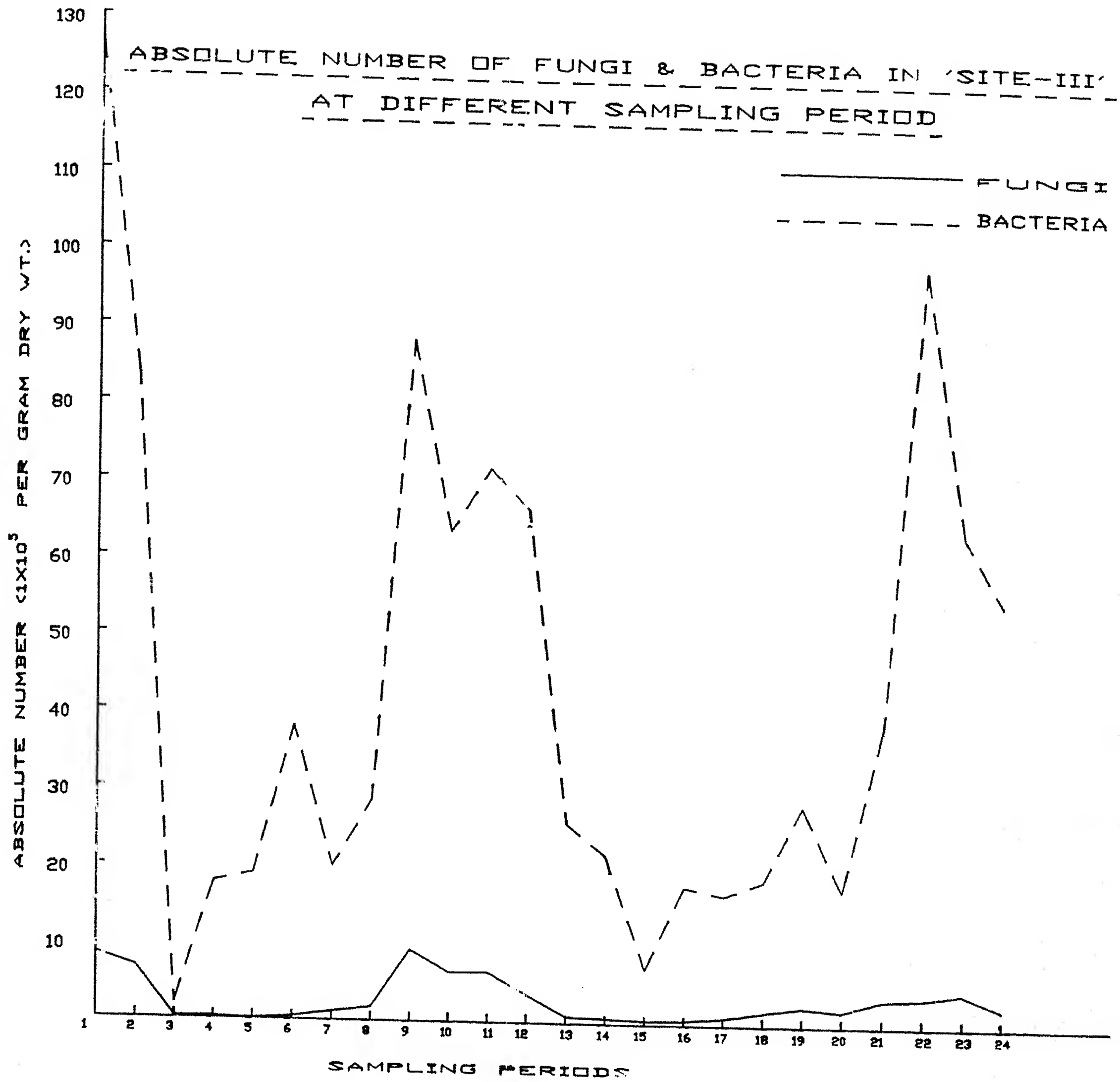
The absolute number of fungal & bacterial species isolated from 'Site III' at different sampling periods are shown in table VII & fig. 7.

The absolute number of fungi was high in Ist sampling with a slight decline in the second record. Then was a sharp decline in number from IIIrd to VIth sampling period, than again rise in number from VIIth & reach to maximum in IXth sampling & this gradually decreases upto XVIth sampling period . From XVIIth sampling the absolute number of fungi again increased gradually up to XXIIIth sampling & in last sampling i.e.XXIVth number of fungi again decreases. Maximum number of fungi were recorded in IXth sampling period. i.e. 9.19×10^5/gm dry wt. of cotton & minimum number of fungi were recorded in Vth sampling i.e. 0.08×10^5/gm dry wt. of cotton.

TABLE - VII

Absolute number of Fungi and bacteria in 'Site - III' at differnet sampling periods (expressed as 1×10^5 per gram dry weight of cotton).

Sampling period	Absolute No. of fungi	Absolute No. of bacteria.
I	8.34	127.9
II	6,67	83.9
III	0.24	03.0
IV	0.28	18.9
V	0.08	20.0
VI	0.42	39.2
VII	1.12	21.0
VIII	1.75	29.6
IX	9.19	89.2
X	6.32	64.6
XI	6.41	72.9
XII	3.62	67.6
XIII	0.90	27.0
XIV	0.68	22.9
XV	0.50	08.0
XVI	0.59	18.9
XVII	0.98	17.8
XVIII	1.77	19.7
XIX	2.47	29.6
XX	2.00	18.5
XXI	3.50	40.0
XXII	3.74	99.3
XXIII	4.45	64.9
XXIV	2.45	56.0


ABSOLUTE NUMBER OF FUNGI & BACTERIA IN 'SITE-III'
AT DIFFERENT SAMPLING PERIOD
FUNGI
BACTERIA
ABSOLUTE NUMBER (1X10^5 PER GRAM DRY WT.)
130
120
110
100
90
80
70
60
50
40
30
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
SAMPLING PERIODS

(FIGURE-7)

The monthly analysis reveals that in Site III, the effect of atmospheric temperature was less as compared to the effect at site I & II . The absolute number of fungi was high during March & April . In March it reached maximum level, and in January lowest number of fungi were isolated. In May & June number of fungi decreased.

The bacterial absolute number followed atmost the same trend like that of fungi table VII & fig. 7 showes that absolute number of bacteria from the initial sampling declined up to IVth sampling,. thereafter increased upto IXth sampling stage & this number declined gradually up to XVth sampling period & than considerably increased upto XXIIth sampling reached to maximum number i.e. 99.3×10^{5}/gm dry wt. of cotton than again followed by decreased in number upto last sampling i.e. XXIVth.

Qualitative Analysis-

Sixteen species of fungi belonging to nine genera were found colonizing the cotton fiber of site III. The percentage occurence & frequency of these fungi are shown in table VIII & Fig. 8.

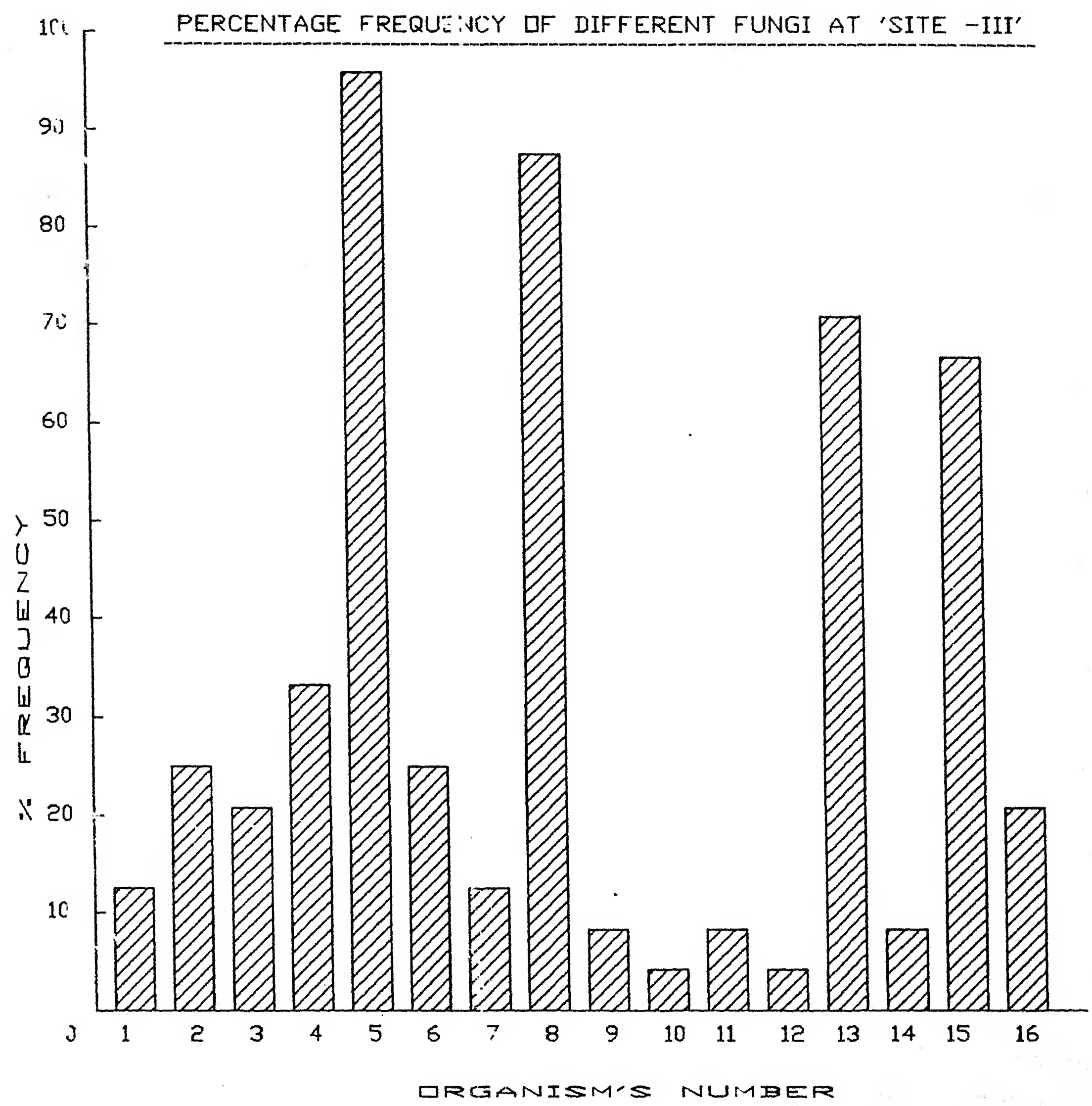
Aspergillus niger, *Aspergillus flavus*, *Penicillium citrinum*, *Rhizopus nigricans*, *Aspergillus oryzae*, *Asprgillus ustus*, *Aspergillus sulphureus*, showed

TABLE - VIII

PERCENTAGE COLONIZATION & FREQUENCY OF DIFFERENT FUNGI 'SITE III' AT DIFFERENT SAMPLING PERIOD

I) SAMPLING PERIOD AT INTERVEL OF 15 DAYS
II) INCUBATION TEMPERATURE 32 ± 2°C

S.No.	ORGANISMS	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	FREQUENCY
1	Alternaria alternata	-	-	-	-	-	-	-	8	67	-	-	-	12.5	16.7	-	-	-	-	-	-	-	-	-	7.2	12.5%
2	Aspergillus sulphureus	-	-	-	33	25	-	-	-	-	5.4	-	-	-	-	-	-	-	5.6	-	5	17.4	-	-	-	25%
3	Aspergillus fumigatus	-	-	-	-	-	-	-	-	-	-	4.5	-	-	-	-	-	-	5.6	8.4	-	-	-	7.7	7.2	20.8%
4	Aspergillus ustus	-	-	-	-	-	-	-	-	6.7	5.4	4.5	13.3	-	-	-	16.6	-	-	8.4	-	-	-	3.8	-	33.3%
5	Aspergillus niger	66.7	75	25	33	50	33	38	46.2	40	40	27.6	13.3	25	33.3	66	33.3	40	44.4	37.5	15	52.4	30.4	46.2	50	95.8%
6	Aspergillus nidulans	-	-	25	-	-	-	-	-	-	2.7	-	-	-	-	-	-	-	-	-	5	-	-	-	-	25%
7	Aspergillus oryzae	-	-	-	-	25	12.5	-	8	-	2.7	9.1	-	-	-	-	-	10	-	-	-	-	4.4	-	-	12.5%
8	Aspergillus flavus	16.7	-	50	-	-	-	37.5	15.4	33.2	33	22.7	53.3	12.5	33.3	40	16.6	30	22.2	20.8	5	38.1	26.1	23	14.3	87.5%
9	Aspergillus terreus	-	-	-	-	-	-	-	-	-	-	-	-	12.5	-	-	-	-	-	4.2	-	-	-	-	-	8.3%
10	Basidiobolus sp.	-	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.2	-	-	-	-	-	4.2%
11	Chaetomiun globosum	-	-	-	-	-	-	-	-	-	-	4.5	-	-	-	-	-	-	-	-	-	-	-	-	-	8.3%
12	Mucor Hydrophilus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.6	-	-	-	-	-	-	4.2%
13	Penicillium citrinum	16.7	-	-	33.4	-	-	12.5	15.4	6.7	5.4	13.6	6.7	-	-	-	16.7	20	5.6	8.4	20	9.5	8.7	7.7	14.3	70.8%
14	Phycomycea sp.	-	-	-	-	-	-	-	-	-	-	-	-	12.5	-	-		-	-	4.2	-	-	-	-	-	8.3%
15	Rhizopus nigricans	-	-	-	-	-	-	13.5	8	6.7	5.4	9.1	6.7	25	16.7	-	16.7	-	11.1	4.2	15	-	4.4	7.8	7.2	66.7%
16	Fusarium oxysporum	-	-	-	-	-	33.4	-	-	-	-	4.5	6.7	-	-	-	-	-	-	4	5	-	4.4	3.8	-	20.8%



PERCENTAGE FREQUENCY OF DIFFERENT FUNGI AT 'SITE -III'
% FREQUENCY
0
10
20
30
40
50
60
70
80
90
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
ORGANISM'S NUMBER

(FIGURE-8)

abundent colonization. Aspergillus niger was found to be dominating in nearly all the sampling. Aspergillus flavus, Aspergillus niger, Penicillium citrinum, Rhizopus nigricans showed most frequent colonization & occurred in high percentage.

Aspergillus ustus, Aspergillus oryzae, Fusarium oxysporum have good percentage colonization though they were isolated only in four or five sampling periods.

Alternaria alternata, Aspergillus fumigatus, Aspergillus terreus, Aspergillus nidulans, Chaetomium globosum, Phycomyces sp. showed low percentage occurence & isolated only in 2 to 4 sampling periods. Mucor hydrophilus & Basidiobolus sp. appeared only once.

Aspergillus niger had highest 95.8% frequency followed by Aspergillus flavus, Penicillium citrirum, Rhizopus nigricans which have 87.5% , 70.08% and 66.7% frequency respectively. Aspergillus ustus had 33.3% frequency. Alternaria alternata, A. sulphureus, A. fumigatus, A. nidulans, A. oryzae & Fusarium oxysporum have percentage frequency between 12% to 25%. Aspergillus terreus, Chaetomium globosum, Phycomyces sp. have 8.3% frequency while Basidiobolus sp. and Mucor hydrophilus had lowest 4.2% frequency.

Site - IV Atmosphere inside spinning chambers.

Quantitative analysis:

The average number of fungi and bacteria isolated from "Site 4" at different sampling periods were recorded in table -IXth. & Fig. 9.

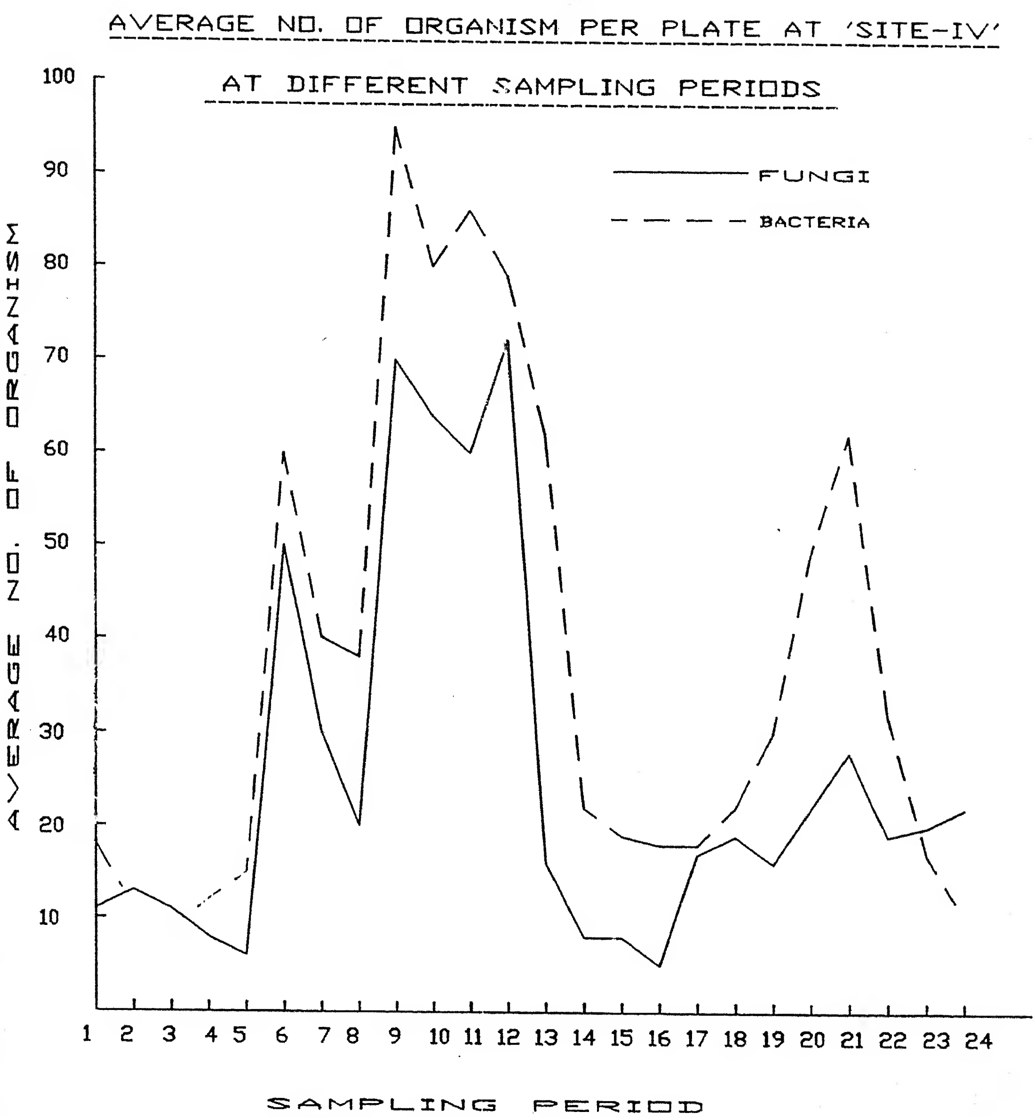
The perusal of data reveals that the average number of fungi during Ist. to IIIrd. sampling periods remained more or less the same then decreased in IVth, and Vth sampling followed by a considerable increase in number in the VIth. sampling. The number than decreased in VIIth VIIIth sampling with slight fluctuations.The number remained very high from IXth.to XIIth. sampling with the peak level at XIIth. sampling. During XIIIth. sampling & there after there was a gradually decline in number of fungi up to XVIth. sampling follwed by a gradual rise upto XXIst. sampling period. This number again decreased during XXIInd sampling & with a slight fluctuation remained almost the same upto last sampling i.e. XXIVth. sampling period Maximum number of fungi were recorded in XIIth. sampling i.e.72 & minimum number of fungi were recorded in XVIth. sampling i.e. 5.

The monthly data revels that the average no. of fungi remained at the highest level during March & April , reaching a maximum during April. During May & June,when the day atmospheric temperature was very high

TABLE IX

AVERAGE NUMBER OF ORGANISM PER PLATE AT `SITE III' ie. FROM AIR INSIDE THE SPINNING CHAMBERS AT DIFFERENT SAMPLING PERIODS.

	SAMPLING PERIOD	NUMBER OF FUNGI	NUMBER OF BACTERIA
1	I	11	18
2	II	13	11
3	III	11	9
4	IV	8	12
5	V	6	15
6	VI	50	60
7	VII	30	40
8	VIII	20	38
9	IX	70	95
10	X	64	80
11	XI	60	86
12	XII	72	79
13	XIII	16	62
14	XIV	8	22
15	XV	8	19
16	XVI	5	18
17	XVII	17	18
18	XVIII	19	22
19	XIX	16	30
20	XX	22	49
21	XXI	28	62
22	XXII	19	32
23	XXIII	20	17
24	XXIV	22	12



AVERAGE NO. OF ORGANISM PER PLATE AT 'SITE-IV'
AT DIFFERENT SAMPLING PERIODS
FUNGI
BACTERIA
AVERAGE NO. OF ORGANISM
100
90
80
70
60
50
40
30
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
SAMPLING PERIOD

(FIGURE 9)

i.e. 42 degree C, the average number of fungi decreased. During February, August, September & October, the average number of fungi were though less than March and April but remained at a higher level as compared to November, December, January, May, June & July. Fig - 9 table IXth.

The average number of bacteria isolated during all sampling periods from air inside the working unit of mill was high as compared to fungi. This might be due to large number of worker involved in these chambers.

From Ist. to Vth. sampling less number of bacteria were present & then in VIth. sampling number of bacteria increased considerably with a decline in VIIth. & VIIIth. reached a maximum level at IXth. sampling. This gradually decreased up to XVIIth. sampling period followed by increase in number from XVIIIth. sampling upto XXIst. sampling, thereafter again the average number of bacteria decreased upto the last i.e. XXIVth. sampling.

Qualitative analysis:

Sixteen species belongs to nine genra were found in the atmosphere of site "number 4".

The percentage occurance and frequency of

TABLE X

PERCENTAGE COLONIZATION & FREQUNCY OF DIFFERENT FUNGI AT 'SITE 4'

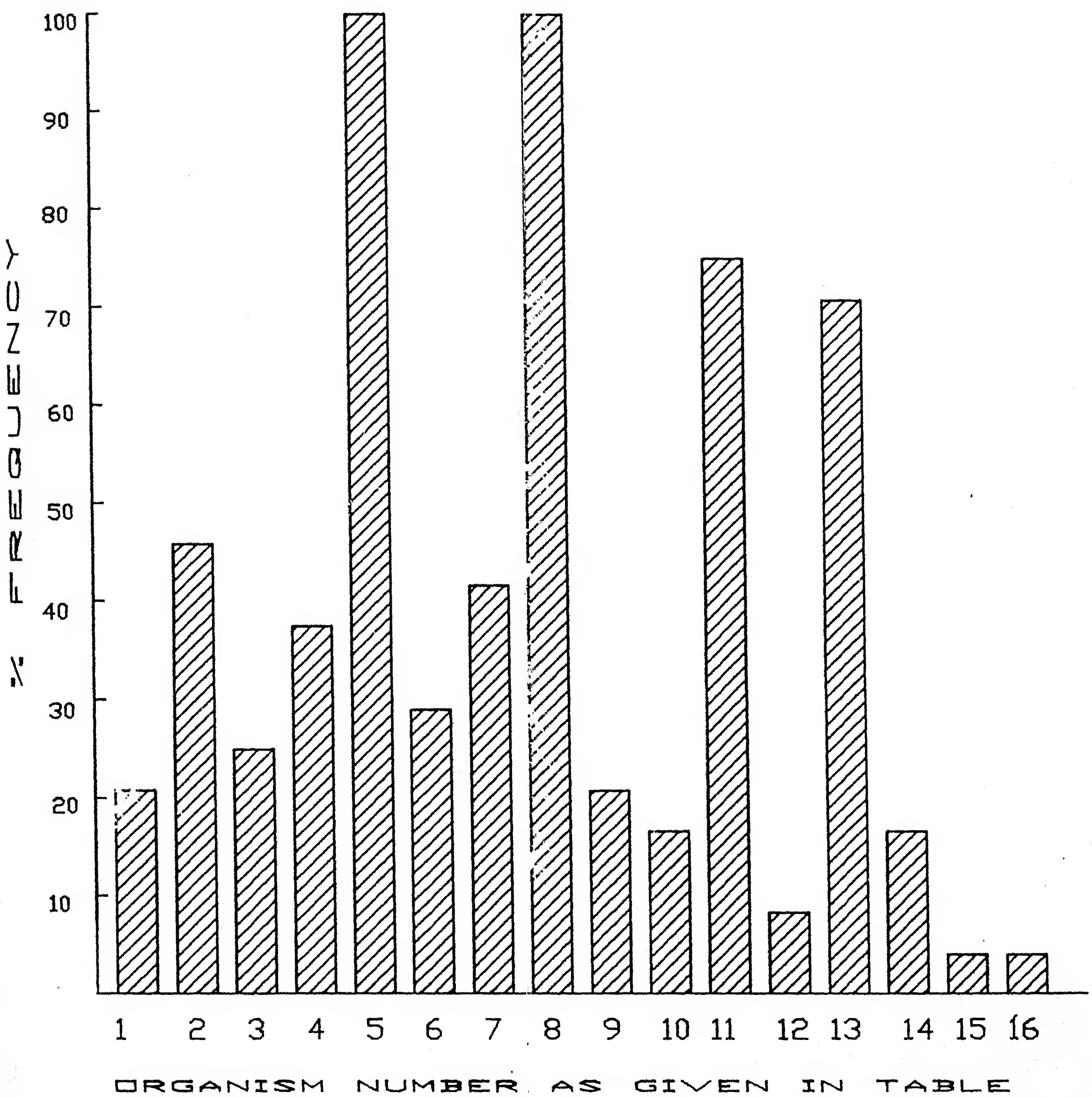
AT DIFFERENT SAMPLING PERIODS

I) SAMPLING PERIOD AT INTERVEL OF 15 DAYS

II) INCUBATION TEMPERATURE 32 ± 2°C

S.NO.	ORGANISMS	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	FREQUENCY
1	Alternaria alternata	-	-	-	-	-	8	-	-	2.8	5	2	-	-	-	-	-	6	-	-	-	-	-	-	-	20.8%
2	Aspergillus sulphureus	9	15	-	-	-	6	-	-	7.1	6	2	8	12.5	-	-	-	12	21	-	-	-	16	5	-	45.8%
3	Aspergillus fumigatus	-	-	-	-	-	2	-	-	-	3	3	-	-	-	-	-	-	10	-	-	4	16	-	-	25.0%
4	Aspergillus ustus	-	-	18	-	-	6	-	10	-	3	3	3	-	-	-	-	-	10	-	-	-	5	10	-	37.5%
5	Aspergillus niger	36	38	54	37.5	66	36	36	30	38	39	35	33	31	50	37	60	29	36	31	36	42	42	35	36	100%
6	Aspergillus nidulans	-	-	-	-	-	-	-	5	2.8	3	-	3	6.2	-	-	-	-	-	-	5	-	-	-	5	29%
7	Aspergillus oryzae	-	-	-	12.5	-	6	-	10	1.4	5	5	3	-	-	-	-	5	-	-	-	4	-	-	9	41.6%
8	Aspergillus flavus	27	23	18	12.5	33	20	30	20	20	21	30	27	37	38	38	20	23	11	31	27	28	21	40	27	100%
9	Aspergillus terreus	-	8	-	-	-	-	-	-	2.8	-	3	-	-	-	-	-	-	-	-	9	-	-	-	9	20.8%
10	Chaetomium globosum	-	-	-	-	-	-	3.3	-	4.2	-	-	-	-	-	-	-	-	-	6	-	-	-	-	5	16.6%
11	Penicillium citrinum	18	15	-	12.5	-	8	16	10	8.5	7.8	13	5.5	13	-	-	20	12	-	19	-	14	5.2	10	5	75%
12	Phycomyces sp	-	-		-	-	-		5	-	1.5		-	-	-	-	-		-	-	-	-	-		-	8.3%
13	Rhizopus nigricans	90	-	9	25	-	4	13	5	5.7	4.6	3	6.9	-	-	25	-	5.8	10	16	22	7	-	-	5	70.8%
14	Fusarium oxysporum	-	-	-	-	-	4	-	5	1.4	-	-	1.3	-	-	-	-	-	-	-	-	-	-	-	-	16.6%
15	Trichoderma viridi	-	-	-	-	-	-	-	-	-	-	-	1.3	-	-	-	-	-	-	-	-	-	-	-	-	4.0%
16	Verticillum alboatrum	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	5	-	4.0%

PERCENTAGE FREQUENCY OF FUNGI AT 'SITE-IV'



(FIGURE -10)

these fungi are shown in table Xth. & Fig.10.

Aspergillus niger, Aspergillus flavus, A.sulphureus, A.nidulans, A.oryzae, A.ustus, Penicillium citrinum, Rhizopus nigricans showed good percentage colonization.

Aspergillus niger was found to be most dominating in all sampling periods. Aspergillus flavus, Penicillium citrinum & Rizopus nigricans were next to A.niger in showing frequent colonization & percentage frequency.

Alternaria alternata, Aspergillus fumigatus, Aspergillus ustus, Aspergillus terreus, chaetomium globosum, showed less percentage occurence & were isolated only in 4-5 sampling.

Fusarium oxysporum, Trichoderma viridi, Verticillum alboatrum showed very low percentage colonization & were isolated only in 1-2 sampling.

A. niger & A.flavus had the highest 100% frequency followed by Penicillium citrinum & Rhizopus nigricans, which had 75% & 70.8% frequency respectively. The percentage frequency of A.oxyzae, A.Ustus ranged between 38% to 49% & of Alternaria alternata, Aspergillus fumigatus, A.nidulans, Chaetomium globosum, Fusarium oxysporium ranged between 16-25% frequency.

Trichoderma viridi, *Phycomyces sp*, *Verticillum alboatrum* had the minimum percentage frequency i.e. between 4% to 8.3%.

Site V :Atmosphere outside the spinning mill (Working unit).

Quantitative analysis : The average number of fungi & bacteria isolated from "site 5" at different sampling period are recorded in table - XIth. & Fig. 11.

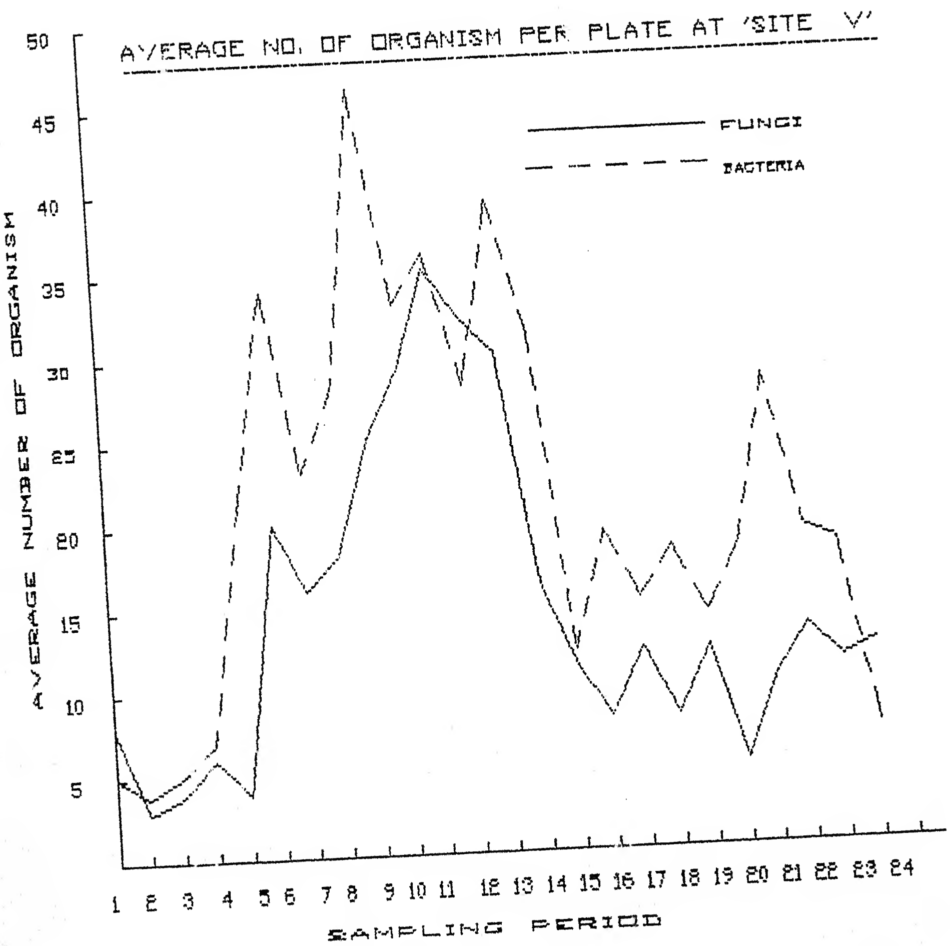
The data recorded in table XIth. shows that average number of fungi per plate from Ist. to Vth. sampling were very low & remained more or less the same. Then increased considerably in the VI sampling with a slight decreased in the VIIth. & VIIIth. sampling, again gradually increased to a maximum level on the XIth. sampling. From XIIth. sampling onwards the average number gradually declined. Thereafter up to the last fungi were recorded in XIth. sampling i.e. 35 & minimum no. of fungi were recorded in IInd. sampling period i.e. 3.

The monthly data reveals that number of fungi increased during March & April when atmospheric tempreture in day was 30 - 32 degree C & fast winds were blowing. During November, December & January when atmospheric tempreture was very low, the average number of fungi remained at lowest level. During May & June

TABLE XI

AVERAGE NUMBER OF ORGANISM PER PLATE AT `SITE V' ie. FROM AIR OUTSIDE THE MILL AT DIFFERENT SAMPLING PERIODS.

SAMPLING PERIOD	NUMBER OF FUNGI	NUMBER OF BACTERIA
I	8	5
II	3	4
III	4	5
IV	6	7
V	4	21
VI	20	34
VII	16	23
VIII	18	28
IX	25	46
X	29	33
XI	35	36
XII	32	28
XIII	30	38
XIV	16	31
XV	11	12
XVI	8	19
XVII	12	15
XVIII	8	18
XIX	12	14
XX	5	18
XXI	10	28
XXII	13	19
XXIII	11	18
XXIV	12	7


AVERAGE NO. OF ORGANISM PER PLATE AT 'SITE V'
FUNGI
BACTERIA
AVERAGE NUMBER OF ORGANISM
50
45
40
35
30
25
20
15
10
5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
SAMPLING PERIOD

(FIGURE -11)

average no. of fungi remained at lower level.

The bacterial population was increased from Vth.to IXth. sampling & reached to maximum level in the IXth. sampling. From I-V sampling average number of bacteria were lowest followed by increase in number upto IXth sampling.After that number of bacteria slightly & gradually decreased upto XIVth. sampling period. From XVth. sampling number of bacteria again declined except in the XXIst. sampling when the average number of bacteria was comparatively high,the number remained almost the same however with slight fluctuations.

Qualitative analysis :

The percentage colonization & frequency of different fungi of "site 5" at different sampling period were recorded in table XIIth. & Fig. 12.

Twenty species belonging to ten genera were found in the air, outside the working unit. Aspergillus niger, A. flavus, Penicillium citrininum, Rizopus nigricans showed abundent colonization throughout the sampling. Aspergillus niger was found to be most dominating species during various sampling period.

Aspergillus niger, A. flavus, Penicillium citrinum, Rhizopus nigricans showed most frequent

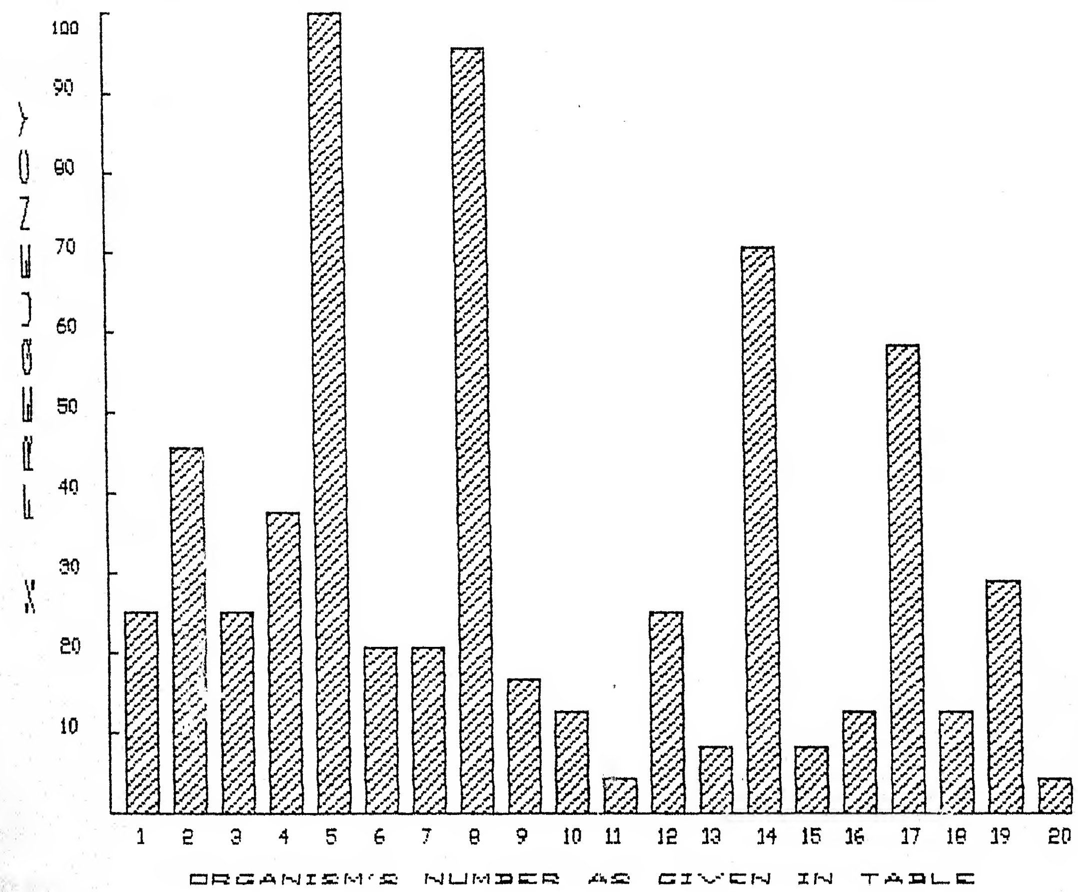
TABLE XII

PERCENTAGE COLONIZATION & FREQUNCY OF VARIOUS FUNGI AT 'SITE V'
AT DIFFERENT SAMPLING PERIODS

I) SAMPLING PERIOD AT INTERVEL OF 15 DAYS
II) INCUBATION TEMPERATURE 32 $\pm$ 2°C

S.NO	ORGANISM	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	FREQUENCY
1	Alternaria alternata	-	-	-	-	-	-	-	-	8	3.4	-	3.1	3.3	-	-	-	-	-	8.3	20	-	-	-	-	25%
2	Aspergillus sulphureus	-	-	25	-	-	-	-	11	4	6.8	8.5	6.2	3.3	-	-	-	25	25	-	-	-	-	18	8.3	45.8%
3	Aspergillus fumigatus	-	-	-	-	-	-	-	5.5	8	-	-	9.3	-	14.5	-	-	-	-	16.6	-	-	-	9	-	25%
4	Aspergillus ustus	25	-	-	-	-	10	-	-	-	6.8	8.5	3	-	12.5	-	-	16.6	12.5	-	-	20	-	-	-	37.5%
5	Aspergillus niger	25	33.3	50	33.3	50	25	18.7	22.2	28	27.5	25.7	25	33.3	31.2	36	25	25	25	41.6	20	30	38.4	27.2	33.3	100%
6	Aspergillus nidulans	12.5	-	-	-	-	5	-	-	-	-	-	6.2	-	-	9	-	-	-	-	-	10	-	-	-	20.8%
7	Aspergillus oryzae	-	-	-	-	-	-	-	11	-	-	6.2	-	-	6.2	-	-	8.3	-	-	-	-	-	-	8.3	20.8%
8	Aspergillus flavus	25	66.6	-	16.6	25	20	12.5	16.6	20	13.7	17.1	15.6	20	-	27.2	37	25	12.5	16.6	40	40	38.4	9	25	95.8%
9	Aspergillus terreus	-	-	-	-	-	-	6.2	-	-	-	-	3.1	6.6	-	-	-	-	-	-	-	-	15.3	-	-	16.6%
10	Aspergillus atropurpureus	-	-	-	-	-	-	-	-	-	3.4	28	-	-	-	9.0	-	-	-	-	-	-	-	-	-	12.5%
11	Basidiobolus sp.	-	-	-	-	-	-	-	-	-	-	-	-	3.3	-	-	-	-	-	-	-	-	-	-	-	4.2%
12	Chaetomium globosum	-	-	-	-	-	5	-	-	4	-	-	6.2	6.6	6.3	-	-	-	-	-	-	10	-	-	-	25%
13	Cladosporium sp.	-	-	-	-	-	-	-	5.5	-	3.4	-	-	-	-	-			-	-			-	-	-	8.3%
14	Penicillium citrinum	12.5	-	-	33.3	-	10	6.2	-	12	13.7	14.2	12.5	10	18.7	9.0	-	-	25	8.3	20	-	7.6	18	16.6	70.8%
15	Penicillium sp.	-	-	-	-	-	10	-	-	-	-	-	-	-	6.2	-	-	-	-	-	-	-	-	-	-	8.3%
16	Phycomyces sp.	-	-	-	-	-	-	-	-	-	6.8	-	-	3.3	6.2	-	-	-	-	-	-	-	-	-	-	12.5%
17	Rhizopus nigricans	-	-	25	-	25	5	-	5.5	8	10.3	6.5	6.2	3.3	-	9.0	12.5	-	-	8.3	-	20	-	18	-	58.3%
18	Trichoderma viridi	-	-	-	-	-	-	-	5.5	-	3.4	-	-	6.6	-	-	-	-	-	-	-	-	-	-	-	12.5%
19	Fusarium oxysporum	-	-	-	-	-	10	-	11	4	-	-	3.1	-	-	-	25	-	-	8.3	-	-	-	-	8.3	29.1%
20	Fusarium moniliforme	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.2%

PERCENTAGE FREQUENCY OF VARIOUS FUNGI AT 'SITE -V'



(FIGURE-12)

colonization & occur in higher percentage.

Alternaria alternata, *Aspergillus sulphureus*, *A. fumigatus*, *A. ustus*, *A. nidulans*, & *Fusarium oxysporum* appeared in 4-5 sampling during earlier or later isolations.

Aspergillus oryzae, *A. tereus*, *A. atopurpureus*, *Chaetomium globosum*, *Phycomyces sp.*, *Trichoderma viridi* showed low percentage occurence & were isolated only in 3-4 sampling.

Basidiobolus sp., *Cladosporium sp.*, *Penicillium sp.* & *Fusarium moniliforme* appeared only in 1 or 2 sampling.

Aspergillus niger had 100% frequency followed by *Aspergillus flavus*, *Penicillium citrinum* & *Rhizopus nigircans*. Which had 95.8%, 70.8% & 58.3% frequency respectively.

Aspergillus sulphureus had 45.8% frequrncy. The percentage frequency of *Alternaria alternata*, *Aspergillus fumigatus*, *A. ustus*, *A. orzae*, *A. nidulans* & *Fusarium oxysporum* ranged between 20 - 30% & that of *Aspergillus terreus*, *A. atropurpureus*, *Phycomyces sp.* & Trichoderma viridi range between 12 - 16%.

Clodosporium sp. & *Penicillium sp.* had 8.3% frequency, *Basidiobolus sp.* & *Fusarium moniliforme* had the lowest frequency of 4.2%.

During isolation of microorganism from different sites of spinning mill some pathogenic bacteria were frequently isolated. These were **Staphylococcus aureus**, **Streptococcus pyogens**, **Bacillus sp.**, **proteus sp.** & **Mycobacterium tuberculosis**. These bacteria were isolated on, Nutrient agar media, Blood agar media & Lowenstein Jensen media & were recultured for further studies.

SECTION II

SUB SECTION B

COMPARATIVE CELLULOLYTIC ABILITY

CHAPTER - 7

INTRODUCTION

The ability to utilize cellulose is considered essential for saprophytic fungi (Melin 1948). The field of utlization of cellulose has attracted many workers, Reese (1947); Siu (1951); Reese & Levinson (1952); Garrett (1962, 1963, 1966); Hogg (1966); Rai (1969, 1970); Fergus (1969); Dwivedi & Singh (1974); These have suggested that the successful saprophytic colonization of a fungus largely depends upon its cellulolytic ability. This is reflected by the amount of cellulose utilised by the organism. In most cases fungi have been studied in pure culture. Tribe (1957, 1960) & Garrett (1962) have however, investigated the decomposition of cellulose in soil. While it may be argued that what occur in petridishes in the laboratory on chemically defined medium is different from what occur in the soil in competition with other organisms.Repeated isolation of particular fungi from litter at various stages of decay & demonstration of their cellulolytic ability in pure culture must at least prove its potential for competitive saprophytic survival in litter (Chesters 1960).

Siu(1951) has given a list of cellulolytic fungi isolated from competitive conditions on cotton fabrics. White <u>et. al.</u>, (1948); have also demonstrated

the ability of fungi to degrade cellulose. They also found that **Alternatia pullulans** although isolated from exposed cotten fabrics & many decaying plant material is not cellulolytic. Its persistence on leaves may be through its ability to break down pectic substances (Smit & Wieringa 1953).

The cellulolysis adequacy index is defined by Garrett (1966); as the respiration rate of a fungal colony growing on cellulose per unit of colony extension. An increase in the index associated with an increase in cellulolysis rate chiefly results from an increase in density of the mycelium through a higher rate of hypal branching. For the purpose of this particular inquiry, the method of estimating rate of cellulosysis through dry weight loss of the inoculated filter paper seems to be satisfactory. The method is less satisfactory for estimating the actual percentage of cellulose decomposed, because this exceeds percentage loss in dry weight by proportion corresponding to dry weight of mycelium synthesized (together with that of any products of cellulolysis not yet absorbed by the mycelium. Hogg (1966) suggested that comparision should perhaps be made between high cellulolytic activity together with a high dry weight production; lower cellulolvtic activity & low dry weight production; low cellulolytic activity & high dry

weight production. The two groups (i) saprophytes have higher cellulolytic ability & also grow rapidly (ii) Fungi grow slowly & have a lower cellulolytic ability Macer (1961) points out that **Cercosporella herpotrichoides** with the lowest rate of cellulose decomposition also should have the longest period of saprophytic survival in burried straws. He regarded such an economic rate of substrate utilization as tending to conserve substrate reserves & leading to a longer period of survival.

Rai (1970) found that the primary colonizers utilised more cellulose in comparision to others following them & had a higher dry weight production of hyphae. Secondary colonizers utilised cellulose less in comparison to the foregoing with a fair dry weight production of mycelium.

As currenTly understood (Fergus 1969) the utilization of native cellulose depends upon the ability of the organism to produce two types of enzyme designated as C_1 & Cx. The C_1 enzyme act on crystalline cellulose in such a way that subsequent action by Cx enzyme becomes possible.The Cx enzyme is a complex of enzyme hydrolysing the beta 1,4-glucosidic bonds in the cellulose molecules. The Cx enzyme has the ability to degrade cellulose derivatives such as soluble carboxymethyl cellulose and celluloses.

From the above consideration the fungi which were found to be most frequent & dominated during the investigation in sub- section"A", were investigated as regards to their ability to utilise cellulose in pure culture. The method employed were that of Garrett (1962) & the composition of the medium employed was that of Hogg (1966).

CHAPTER - 8

EXPERIMENTAL

In these experiments, the comparative cellulolytic ability of the dominant fungi reffered as above & isolated in sub-section "A" were studied i.e. Aspergillus niger, Aspergillus flavus, Penicillum citrinum & Rhizopus nigricans were selected to observe their comparative ability to utilise cellulose in pure culture.

The method of Garrett (1962); Reese & Levinson (1952) were followed & the composition of medium was of Hogg (1966) with the modification that filter paper were replaced with cotton as sole carbon source. Loss of weight of cotton reflected the amount of cellulose respired & does not include the proportion converted into fungal substance.

The medium used was of following composition.

NH_4NO_3	-	0.1%	$FeSo_4$	-	0.001%
KH_2Po_4	-	0.1%	Yeast extract	-	0.003%
$MgSo_4 7H_2O$	-	0.05%	Cotton	-	1.000%

(Collected from Spinning mill)

pH of medium was 6.1 - 6.3

To each of a number of 500 ml erlchmeyer flask was added about 1.0gm. of dry cotton suspended in 100ml. of medium. The flasks were autoclaved at 15 lbs pressure/Sq. inch. for 15 min. & then inoculated. In

each experiment three replicates were inoculated with a 7mm agar disc of the fungus & three flask were left unioculated as control. The flasks were incubated at 28 degree C. After 25 days flasks were sampled. The cotton from each flask was removed washed with distilled water & then dried at 80 degree C till constant weight. Dry weight loss of cotton in each flask was noted. The pH was measured before inoculation. The results were recorded in table XVIII.

Table - XVIII

Loss of weight of cellulose (cotton) by fungi. after 25 days of incubation.

S.No.	Organisms	Loss (mg) in wt. of cotton	% loss in wt. of cotton
1	Aspergillus niger	220	22
2	Aspergillus flavus	125	12.6
3	Penicillium citrinum	108	10.8
4	Rhizopus nigricans	110	11

CHAPTER - 9

RESULT & CONCLUSION

It was found that all the selected fungi were able to utilise varying quantity of cellulose in culture. Aspergillus niger was found to be most active with 22% loss in dry wt. of cotton. It utilised more of cellulose as compared to others. A. niger was followed by A. flavus, Penicilluum citrinum & Rhizopus nigricans successively in their capacity of cellulose utilizations.

Aspergillus flavus was next to A. niger with 12.6% cellulose utilization while Rhizopus nigricans & Penicillium citrinum gave almost the same level of cellulose utilization i.e. 11 & 10.8% respectively.

SECTION II

SUB SECTION C

COMPETITIVE SAPROPHYTIC COLONIZATION

CHAPTER-10

COMPETATIVE SAPROPHYTIC COLONIZATION

INTRODUCTION:-

The term competitive saprophytic ability was introduced by Garrett(1950), Garrett (1956) suggested that in colonization of dead organic matter competative saprophytic ability is one of the main factor.

Garrett (1944) distinguinshed between saprophytic servival and saprophytic colonization. Saprophytic survival is the servival of a parasite in dead host tissue that it originally inveded and occupied while they are still alive. Garrett calls root inhabitants to such form " Competitive Saprophytic colonization" is the invasion of dead tissue in competition with purely saporphytic fungi and other soil microorganism. The true saprophytic servival is a slow mycelial development of a parasite with in the dead host tissue. In the servival the pathogen has to face the competition from the soil micro flora and this competition has been called, as the "Competitive Saprophytic ability"; (Garrett 1956,62,63). He has defined competitive saprophytic ability as "the summation of physiological characteristics that make for success in competitive colonization of dead organic substrates".

He has explained that the out come of any struggle between one particular micro organism and other for the colonization of a substract will depend upon three charactriestices of the micro-organism concerned.

1. Competitive saprophytic ability of the particuler fungus.

2. The inoculum potential.

3. Environmental conditions including the population of competing fungi and other micro-organism.

Garrett (1950) had earlier suggested three factors that are likely to influence the former attribute.

(i) Intrinsic growth rate of fungus and rapid germination of spores.

(ii) Good enzymes producing capacity

(ii) Tolerance with antibiotic produced by others soil microorganism.

Garrett (1950) defined "inoculam potential as the energy of growth of fungus available for colonization of substract at the surface to be colonized. The third important group of factors concerned in Saprophytic colonization is constituted by enviromental conditions including in particuar the number and variety of competitions in the immediate

vicinity of both the particular micro- organism under investigation and the substrate it is about to colonize.

Competitive saprophytic colonization is obiously one of the two forms of colonization (the other being parasitic) and initiation of its studiess associated particularly with DR. S.D.Garrett and his associates (1956, 1963, 1979).

Saprophytic behaviour in case of root infecting fungi in soil has been extensively studied by many workers, among these more notable work has been that of Blair (1943); Sadasivan (1939); Butler (1953a.b.c.) Lucus(1955); Macer (1961); Rao (1959); Wastie (1961) and Upadhyay et. al., (1980,1982), conclusions drawn by these workers and those of Garrett are based mainly on their studies on root affecting fungi i.e.on fungal pathogen's .Thus the behavior of these pathogens with other inhabiting fungi were studied by them . The present author is however not concerned with plant pathogenic fungi but with the saprophytic fungi which had colonized cotton inside the mill. The behaviour of the saprophyts in competition with the other saprophytic inhabitants must be influenced by the same conditions, as they are liable to be effected in the same manner . The saprophytic colonization of fungi in competition with other

saprophytic fungus has not been given much attention.Thus the present work was under taken to study the trend in their saprophytic colonization of the organism which were most frequently found during the isolation studies.

The evolution of the precise technique for estimating "Compititive saprophytic" colonizaton came with the work of Butler (1953a) and subsiquently improved by Lucus (1955)known as "cambridge method," based on already known dilution technique. This method mainly consider the effect of inoculum potential on saprophytic calonization. Other methods have been devised by Park (1958,1959)in an indegendent approach to the same general problem.

Dobbs and Hinson (1953) have given evidence of the widspread occurance in soil of a fungestatic factors inhabiting spores gemmination.

Garrett (1956) used agar plate model of saprophytic competition, suggested that it might be put on more practical systematic use for the investigations of competitive saprophytic colonization . Rao (1958,1959) modified the original "cambridge method." In Rao's technique the test substrate for the competitive Saprophytic colonization was a plate of acidified Czapak-Doxyeast extract agar. Wastie(1961)

designed the cellophane method for investigating the competitive saprophytic colonization of different fungi on agar plate and mainly emphasized on competitive saprophytic ability of the pathogen. Competitive saprophytic ability, deserve more attention because this ability is intrinsic and is one of the genetically determined characteristic of the micro organism.

The success of'cellophane method' depends on the following facts.

(1) That easily diffusaeble nutrients and fungistatic substances passed through cellophane quickly, while the fungal hypae and other micro organism of the soil took time to penetrate the cellophane.

(2) Another advantage of the techinique was that the effect of sample of different site could be conveniently compared with respect to the competitive colonization of the test fungus on agar plates.

(3) The third advantage of this method was that intensity of competition could be increased by lengthening the time between inoculum of sample on the agar plate and eventual inoculation of the test fungus.

In the present work an attempt has been made to estimate 'Saprophytic colonizing capacity of <u>Aspergillus niger</u>, <u>A. flavus</u>, <u>Penicillium citrinum</u> and

Rhizopus nigricans on agar plate. These fungi were those which were found to be most frequent & dominating during our course of study in section II A. The method employed was that of Wasties (1961).

Chapter - 11

EXPERIMENTAL

The method followed was that of wastie (1961) design on the cellophane paper for estimating saprophytic colonization of fungi on agar plate and mainly emphasized on camparative saprophytic ability of the selected organism. Since comparative saprophytic ability is one of the genetically determined characteristic of the organism, therefore it deserves more attention.

In the present study attention has been paid to the saprophytic colonization by the active dyanamic flora of cotton with in the mill . Their saprophytic ability was tested against the microorganism infesting the following two cotton samples.

(a) Microorganism obtaind from the cotton lying in the undergound absorbing channel.

(b) Microorganism obtained from the cotton lying inside the store of the spinning Mill .

The method employed is that of Wastie (1961) The idea of testing the above two different samples was to examine the differential effect of the Microflora which was likely to be differnt.

Procedure

The method followed was that of wastie with the variation that the pH of medium was kept the same as that of the cotton used. Two different cotton, samples as described above were taken and brought to laboratory in Sterile petridishes for inoculation purpose.

Czapks-Dox agar adjusted to same pH as that of the cotton used was plated in sterile pertidishes and kept uninoculated for 24hrs, so that surface film of water could evoparate. This checked the spreading of bacteria on plate. Cellophane paper were cut to fit in to the inner side of petridish, cellophane paper were boiled for 45 minutes to remove the plasticizer & autoclave in petridish for 15 minutes at 15 lb pressure. These were then kept ready for use. The petriplates were inoculated with the help of sterile loop all over the plate with the water suspension prepared by shaking the cotton sample in 250ml round bottom flask with 100 ml sterile water with a mechanical wrist action shaker for 30 minutes. The inoculated plate were covered with sterilized circular cellophane paper, taking care that there were no air bubble between cellophane & agar plate.

These dishes were inoculated with 4mm agar disc of the test fungus in centre. After the dishes were

inoculated for periods of 0 hr, and 24 hrs, control were run in each cases where no inoculation were done on petridishes. All experiments were done in duplicate and dishes were inoculated at 28 degree C. The diameater of growing colonies were measured after every 12 hrs. up to 72 hrs. & plotted against the time taken in both cases with and without inoculum. The degree of suppression was measured by angle between two curves.

CHAPTER - 12

RESULTS & CONCLUSION

Data on growth rates of each test organism are plotted on graph paper separately for 0 hr, 24 hrs. inoculation of the sample underlying the cellophone. The graphs so obtained are presented in Fig. 13, 14, 15 and 16. The degree of suppression in the angle of growth rate of the test organisms are presented in table XIX. The Perusal of these graphs and the tables indicates that in case where sample water suspension was inocularted, the growth of the test fungus showed a pressing effect due to the production of inhibitory substance produced below the cellophane by the underlying organisms. In case of nearly all the test fungi the growth was considerably reduced where sample water suspension was inoculated and incubated for 24 hrs., before inaculating the test organism, it was also observed that the test organism were successful colonizers in the begining but after a lapse of 24 hrs effective amount of inhabiting substance were produced that caused depression in growth rates. Thus there is a direct corelation between the degree of suppression of growth rate and the time interval between the sample and test fungus inoculation.

By comparing the curves of four test fungi it was found that all the four test fungi appeared to be quite succesfull colonizers.

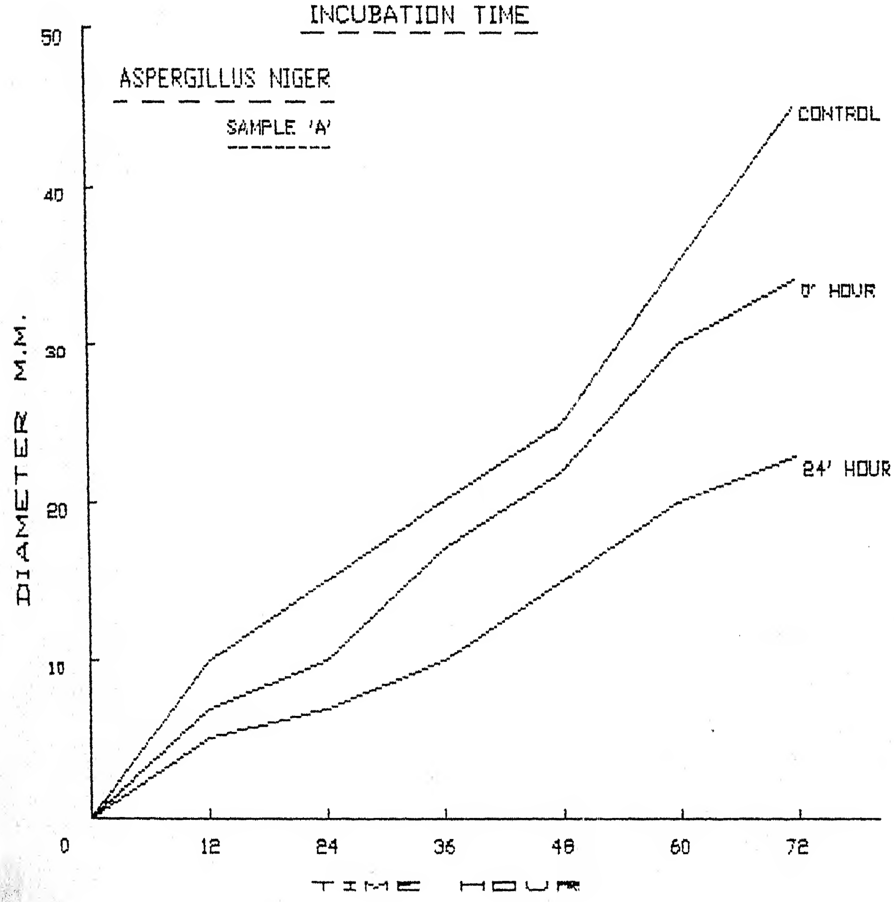
TABLE XIX

DEGREE OF SUPPRESSION IN THE ANGLE OF GROWTH RATE OF THE TEST FUNGI INCUBATION OF SAMPLE WATER SUSPENSION

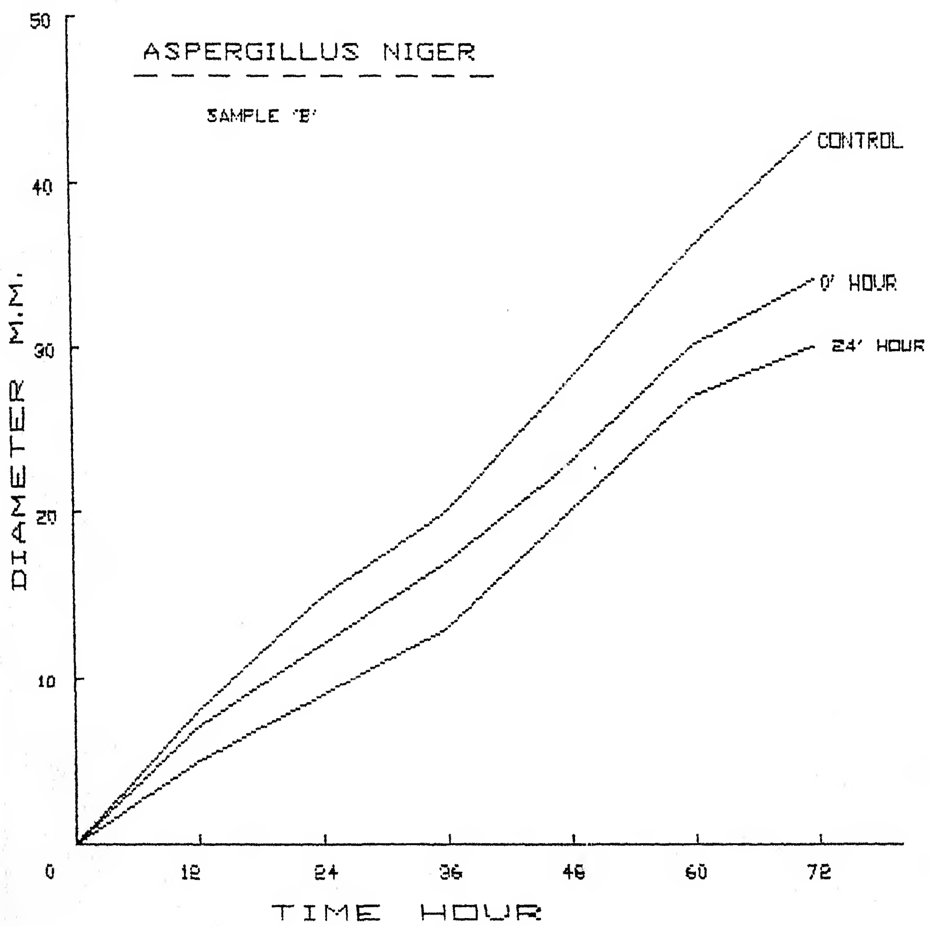
S.No.	ORGANISM	TYPE OF SAMPLE	Ohr.	24 hrs.
1.	Aspergillus niger	Sample A	10	21
		Sample B	8	19
2.	Aspergillus flavus	Sample A	10	19
		Sample B	10	20
3.	Penicillium citrinum	Sample A	9	9
		Sample B	9	10
4.	Rhizopus nigricans	Sample A	6	11
		Sample B	6	11

RADIAL GROWTH OF TEST ORGANISMS AT DIFFERENT

INCUBATION TIME

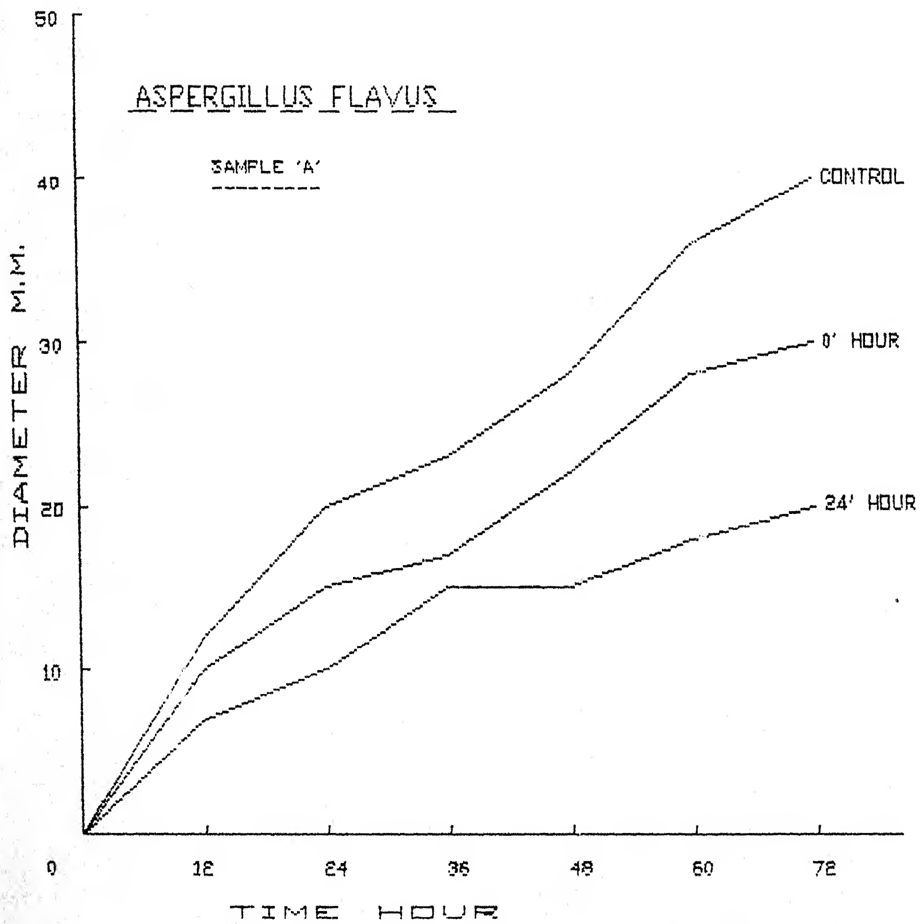


(FIGURE 13 'A')

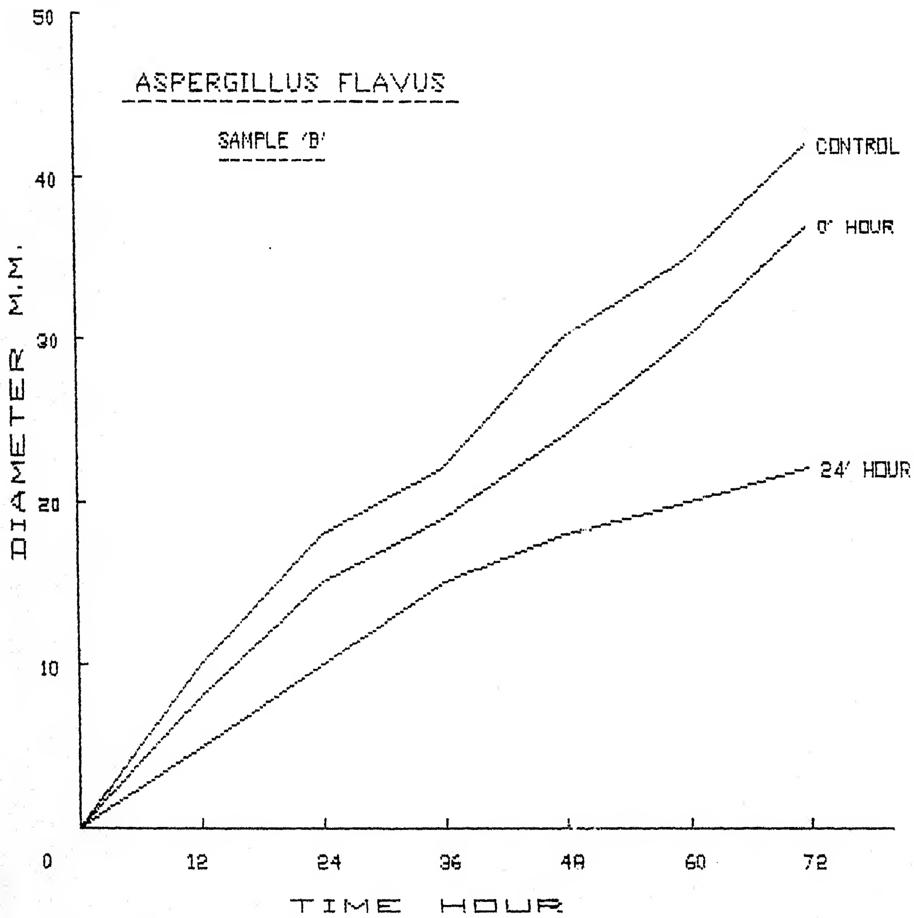


ASPERGILLUS NIGER
SAMPLE 'B'
CONTROL
0' HOUR
24' HOUR
DIAMETER M.M.
50
40
30
20
10
0
12
24
36
48
60
72
TIME HOUR

(FIGURE 13 'B')

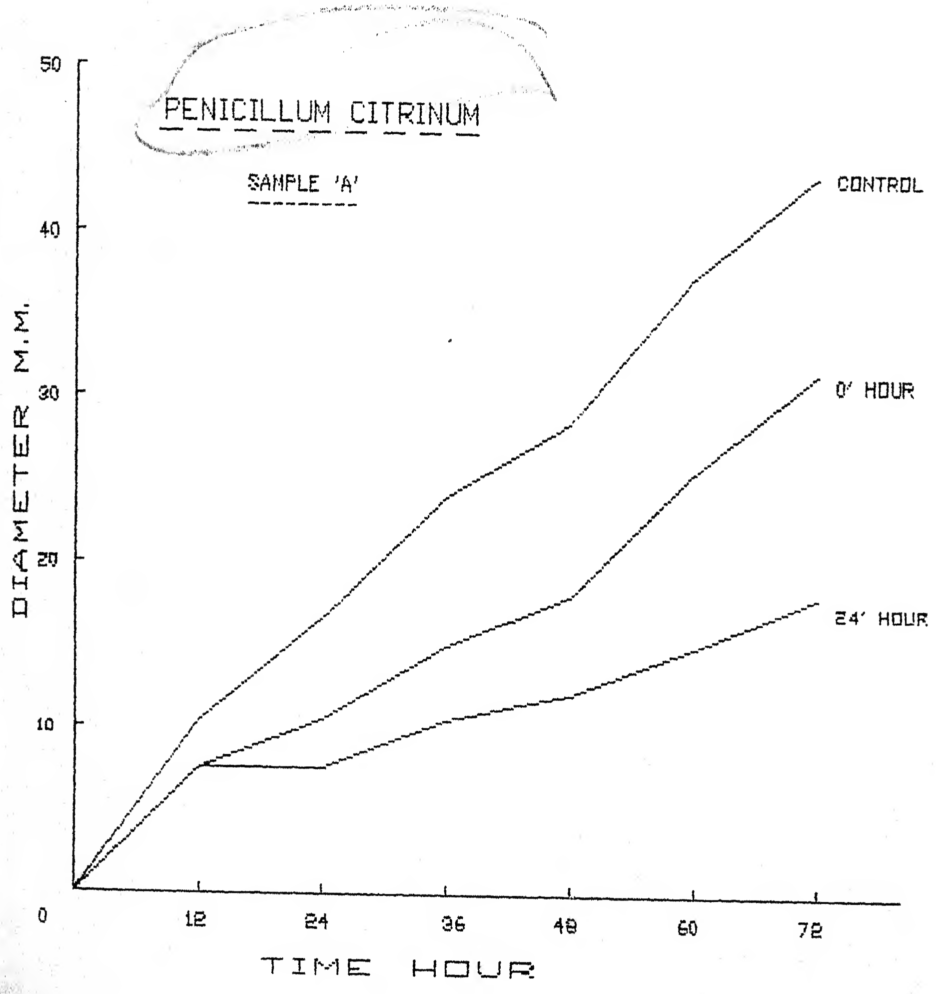

ASPERGILLUS FLAVUS
SAMPLE 'A'
CONTROL
0' HOUR
24' HOUR
DIAMETER M.M.
TIME HOUR
50
40
30
20
10
0
12
24
36
48
60
72

(FIGURE 14 'A')



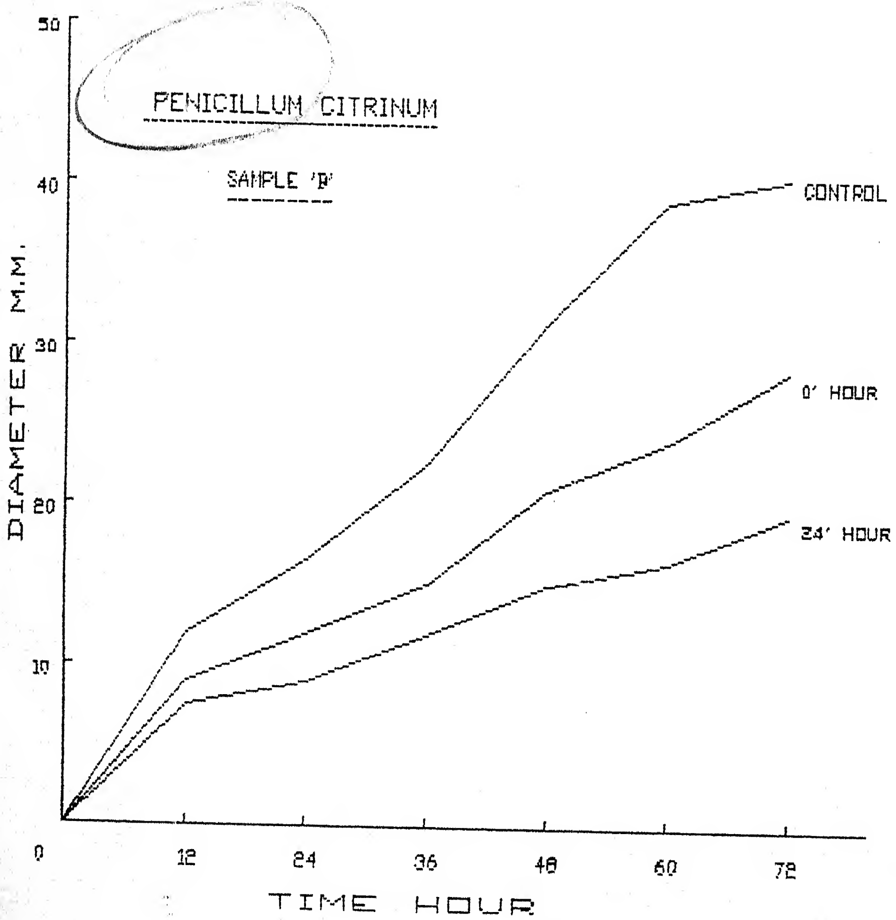
ASPERGILLUS FLAVUS
SAMPLE 'B'
CONTROL
0' HOUR
24' HOUR
DIAMETER M.M.
50
40
30
20
10
0
12
24
36
48
60
72
TIME HOUR

(FIGURE 14 'B')

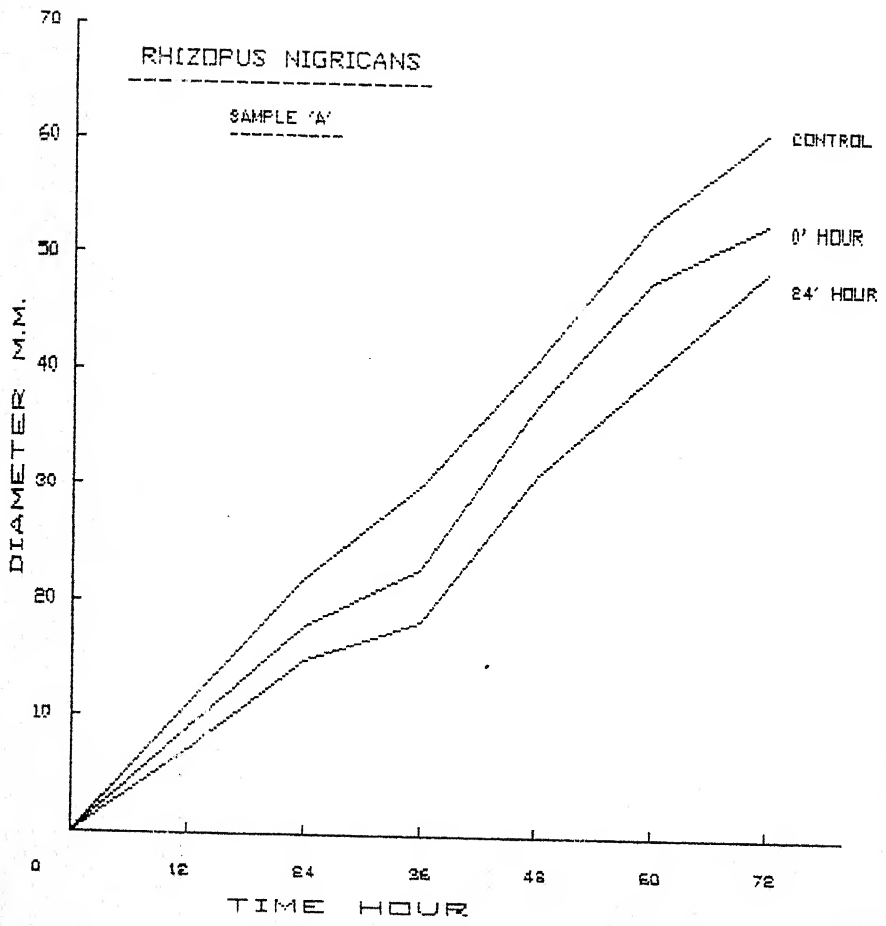


PENICILLUM CITRINUM
SAMPLE 'A'
CONTROL
0' HOUR
24' HOUR
DIAMETER M.M.
TIME HOUR
50
40
30
20
10
0
12
24
36
48
60
72

(FIGURE 15 'A')

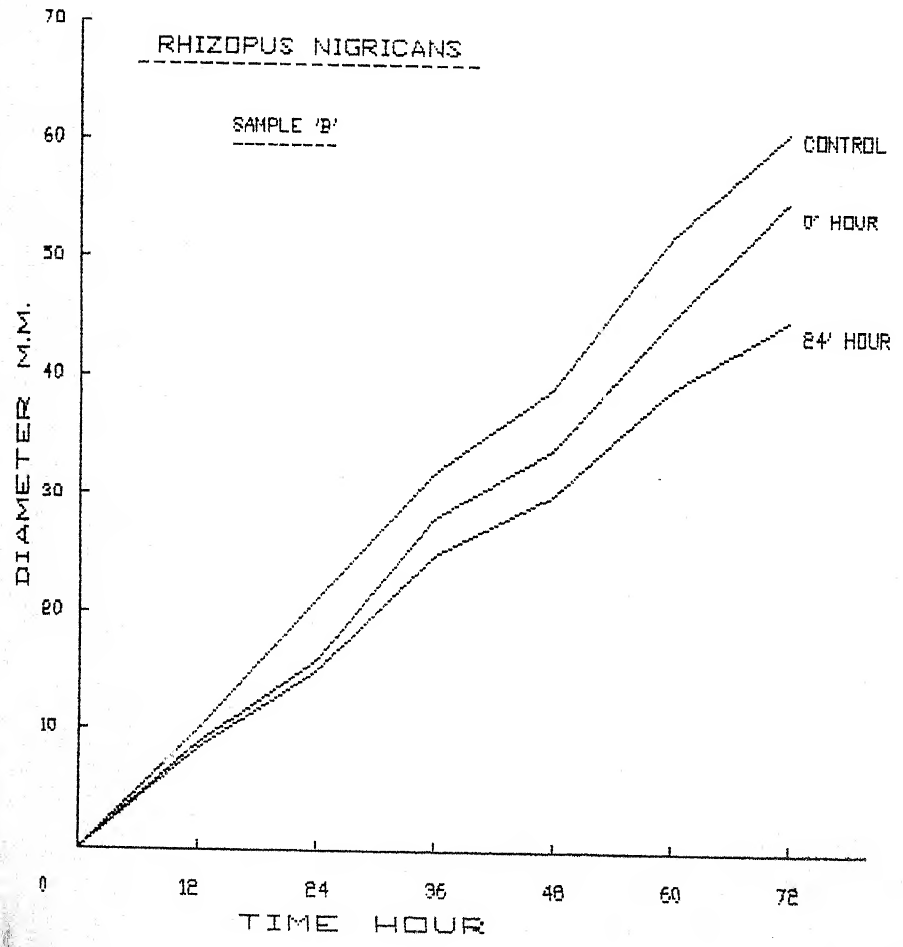

PENICILLUM CITRINUM
SAMPLE 'B'
CONTROL
0' HOUR
24' HOUR
DIAMETER M.M.
TIME HOUR
50
40
30
20
10
0
12
24
36
48
60
72

(FIGURE 15 'B')



RHIZOPUS NIGRICANS
SAMPLE 'A'
CONTROL
0' HOUR
24' HOUR
DIAMETER M.M.
TIME HOUR
0
10
20
30
40
50
60
70
12
24
36
48
60
72

(FIGURE 16 'A')


RHIZOPUS NIGRICANS
SAMPLE 'B'
70
60
50
40
30
20
10
0
DIAMETER M.M.
12
24
36
48
60
72
TIME HOUR
CONTROL
0' HOUR
24' HOUR

(FIGURE 16 'B ')

Coming to the type of samples used (Sample A) obtained from cotton lying in absorving channel, Sample B obtained from cotton of the store). The test fungi gave almost the same results in both case A & B. But in cases of 'A' the deprresion in the angle of growth rate of all the test organism except **Rhizopus nigricans** is greater as compared to that of B.

CHAPTER-13

Summary & Discussion

Sub section A

U.P. State spinning mill of Jhansi is located near the air field and consists of two working units with an average member of 1000 to 1500 workers in the age group of 18 to 50 yrs. working in each shift and two such shifts are involved in day & night working. The workers complain of various health problems like caugh, chest pain, abdominal pain, chest burning and Asthma etc. These workers are exposed to a lot of lint & fibres floating inside the mill within the working chambers. Gupta & Gupta (1984) has reffered the respiratory disorder of cotton mill workers. The cotton fibres floating in air and deposited on the ground harbour a number of micro-organisms which might be of responsible for such ailments. Some organisms must have been incorporated during handling of cotton. In order to enumerate the microflora periodic isolation studies were conducted from cotton sample collected from three different sites by the author viz. absorbing underground channel, store room and the spinning chamber. Samples were taken at 15 days interval and transferred to sterile round bottom flasks with sterile distilled water for shaking. By using wash water for serial dilution plate counts were obtained. Peptone dextrose agar with rose bengal and streptomycin (Martin

1950) for fungi, Soil extract (Allen 1957)/Nutrient agar (Waksman 1961)/Blood agar (Cruickshank et. al., 1971) Lowenstein Jensen medium (Jensen 1955) for bacteria were used for isolations. In addition to the exploration of microbial population on cotton, aerospora survey for bacterial and fungal spores in the air within and outside the mill were also done using gravity petridish method for which sterile petridishes having the above media were exposed in the indoor & outdoor places for various period of time (2 to 20 minutes). The exposed pertidishes were brought back to the lab immediately after the exposure. Observations were made from 2 to 10 days incubation at 32 degree C. Identifications were done using standard taxonomic keys & monograghs reffered in the Appendix II and confirmed from the identified materials from "Kew" England.

The results obtained after 15 days interval for absolute number per gm. dry weight of cotton and percentage occurence have been mentioned in the respective table Site - wise(Table III to XII) these are being discussed below :-

<u>SITE</u>- I <u>Absorbing channel</u> :- The absolute number of fungi isolated from the underground absorbing channel fluctuated throughout the isolation period Table III. Both bacteria and fungi followed the same trend i.e. initially for a few sampling their absolute number

remained almost the same and then declined. During further isolations the absolute number gradually increased & reached the maximum level on the 11th isolation, which decreased considerably on the 16th isolation where it reached the minimum number and then developed into another peak level on the twenty first isolation. There after the number again decreased and reached almost the initial during the last isolation i.e. 24th. In all three peak level were obtained i.e. moderate peak level initially, the highest peak on eleventh isolation & the second highest peak level on the twenty first isolation. The maximum absolute number that was recorded on 11th isolation was 85.5×10^5 /gm dry weight of cotton for fungi & 866.7×10^5 /gm dry weight of cotton for bacteria. The minimum absolute number was recorded on 16th isolation was 2.89×10^5 /gm dry weight for fungi and 37.5×10^5 /gm dry weight of cotton for bacteria. Month wise maximum level was achieved in April and minimum in June.

Qualitatively table IV shows twenty two species belonging to thirteen genera. <u>Aspergillus niger</u>, <u>A. flavus</u>, <u>Penicillium citrinum</u> & <u>Rhizopus nigricans</u> were the most frequent and dominating forms. Highest percentage frequency was of <u>A. niger</u> and <u>A. flavus</u> i.e. 100%, followed by <u>R.nigricans</u> 95.8%, <u>Penicillium citrimum</u> 91.7%, <u>A. ustus</u> 50%,<u>A. sulphureus</u>,

A. fumigatus, *A. terreus*, *Phycomyces Sp.*, *Trichoderma virdi*; *Fusarium oxysporum*, *A. orzyae* and *Alternaria alternata* between 25% to 45%, *Basidiobolus sp.*, *Chetomium globosum*, *cladosporium sp.*, *Penicilliun sp.*, *Paecilomyces varioti*, *Mucor hydrophilus* between 8 to 13% and *Verticilium alboatrum*, *Fusarium solani* had the lowest i.e. 4.2% frequency.

The fluctuations in the absolute number from high to low might be due to the fluctuations in the cimatic conditions of the environment as during the period of investigations the temperature and humidity changed according to the months in which sampling was done. Similarly fluctuations in the frequency of some species and their abrupt absence could also be due to the change in environment.

SITE II Store room :- Table V & fig.5 shows that the absolute number of fungi & bacteria isolated from cotton were much less as compared to the absolute number obtained during the isolations from site I. The absolute number for fungi and bacteria were recorded on the 9th & 12th isolations respectively while the minimum absolute number was on 16th and 15th isolation respectively. The maximum and minimum absolute number obtained for fungi was 37.5×10^{5}/gm dry weight & $.18 \times 10^{5}$/gm dry weight while for bacteria was 395.0×10^{5} and 3.6×10^{5}/gm dry weight of cotton. Month wise the maximum

number was recorded in April and the minimum number during June. A sudden flaring up of microorganism in the month of September as observed in the site 1 was missing in site II.

Qualitatively eighteen sp. belonging to eleven genera were isolated. Highest percentage frequency was again of A. niger and A.flavus i.e. 100 % followed by R. nigricans 87.5% Penicillium citrinum 71.42%, A. sulphureus 45.8%, A. alternata, A. fumigatus, A. ustus, A. oryzae, A. terreus, Chaetomium globosum, Phycomyces sp. between 16% to 13% F.oxysporum, & Cloadsporium sp. 12 to 5% while Verticillium alboatrum & Mucor hydrophilus had lowest i.e. 4.27% frequency Table - VI.

SITE III : The spinning chamber :-Absolute number explored from the composite samples obtained from three chambers situated inside the mill are given in table VII & Fig. 7. Initially the absolute number was high then decreased gradually and then suddenly reached a peak on the 9th isolation which gradually decreased, then again increased and reached another peak on the 22nd and 23rd isolation and then again decreased. The maximum value for fungi was in 9th isolation i.e. 9.19×10^{5}/gm dry weight while for bacteria was on 1st isolation i.e. 127.9×10^{5}/gm dry weight.

The minimum absolute number was obtained for fungi on the 3rd isolation i.e. $.08 \times 10^5$ /gm dry weight and for fungi on the 3rd isolation was 3.0×10^5 /gm dry weight. Compared to the absolute number of organism occuring during the various sampling period of this site the initial high absolute number obtained during November was not found in the earlier sites. This initial high absolute number might be the effect of biotic disturbenses caused by the increased number of manual labour employed during such period of investigations. Monthly analysis shows that during march & April a higher absolute number was found while the lowest number was recorded during the month of January. The working intensity of the mill appears to play a more significant role in flaring up the absoluite number of microorganisms rather then the seasonal variation as the temperature and humidity is being maintained throughout the spinning period within these chambers.

Qualitatively sixteen sp. belonging to nine genera were recorded in the table VIII with *A. niger* having the highest percentage frequency of 95.87%. *A. flavus*, *P. citrimum* and *R. nigricans* had 87.5%, 70.08% & 66.7% respectively. *A. ustus* 33.37% *A. alternata*, *A. sulphureus*, *A. fumigatus*, *A. nidulans*, *A. oryzae*, *F. oxyspoum*, between 12% to 25%. *A. terreus*, *Chaetomium*

globosum sp., Phycomyces sp. 8.3% while Basidiobolus sp. and M. hydrophilus had 4.27 % frequency.

SITE IV "Air inside the spinning mill :- The average number of organisms obtained at this site have been given in table 9 fig.9. The maximum number of organisms were recorded in the IXth isolation and then in XIth and XIIth isolations this number again reduced and reached to a third peak on the XXth and XXIst isolation. Maximum number was 72 for fungi and 95 for bacteria while the lowest was 5 on XVIth isolation of fungi and of Bacteria in the 2nd isolation i.e. eleven.

Qualitative analysis as evident from the data in table X shows that the hightest percentage frequency was of A. niger and A. flavus i.e. 100% followed by P. citrinum 75%, R. nigricans 70.8%, A. sulphureus, A. oryzae, A. ustus, betweem 38% to 49%, Alternaria alternata, A. fumigatus, A. nidulans, Chaetomium globosum, F. oxysporum between 16% to 25% while that of Trichoderma virid, Phycomyces sp., Verticillium alboatrum was between 4% to 8.3%.

SITE V Air out side the spinning unit :- The number of organisms per plate were less as compared to the one observed in the above site. The minimum number of fungi and bacteria were isolated on the 2nd isolation while

the maximum number was observed on the 11th isolation for fungi and 9th isolation for bacteria. For fungi the maximum number and minimum number found were 35 and 3 while for bacteria it was 46 & 4.

The monthly data reveals that the climatic variation has considerably influenced the number of organism appearing per plate . When temperature was very high or very low, the number per plate was reduced, while during March and April when the atmospheric temperature was 30 - 32 degree C the number found per plate increased.

Qualitatively twenty sp. belongiong to ten genera were recorded with the maximum % for*A. niger* i.e. 100%, followed by *A. flavus* 95.8%, *P. citrium* 70.8%, *R.nigricans* 58.3%, *A. sulphureus* 45.8%, *A. alternata*, *A. fumigatus*, *A. ustus*, *A. oryzae*, *A. nidulans* between 20% to 30% while *A. terreus*, *A. atropurpureus*, *Phycomyces sp.*, *Trichoderma, viridi* 12% to 16% *Cladoporium sp.*, *Pencillium sp.* 8.37% and *Basidecbolus sp.*, *F. moniliforme* with the lowest frequedncy i.e. 4.2%.

During isolation pathogenic bacterial forms were also frequently met these were *Staphylococcus aureus*, *Streptococcus*, *pyogenes*, *Mycobacteriun tuberculosis*, *Bacillius sp.* and *Proteus sp.* on Blood

agar Lowenstein Jensen and Nutrient media. These were seperated and stored for future use in further studies.

Bagga (1970); Nigam et. al.,(1959) and Simpson and Marsh, (1969), also isolated fungi from cotton balls & cotton fabrics. Nigam et. al.,(1959) obtained bacteria in the range of 2,000,000 and fungi in the range of 80,000 from cotton fabrics, the results are quite similer to the number obtained by the author. These workers have also isolated **A.flavus**, **A. fumigatus**, **A. nidulans**, **A. niger**, **A. ustus**, **cladosporium**, **herbarm**, **Fusarium moniliforme**, **F. solani**, **Rhizopus**, **stolonifer**, **Trichoderna viridi**, **Verticillum sp.** These results are again similar to the qualitative analysis of the author. Dominance of species of genus "Aspergillus" is in conformitry with reports of Tilak and Chakre,(1979), Jaya Prakash et. al.,(1978) Jaya Prakash and Ramalingan (1981); Santra and Chandra (1981); Mehta and Sandu (1983); **Aspergillus niger** and **A. flavus** were the most common fungal species at both places (indoor and outdoor) (Rashmi Tewary 1991). Sinha et. al.,(1981) have also found the abundance of **A. niger** in the air at Calcutta.

Marsh and Bollenbacher (1949) described fungi concerned in fiber deterioration while Alka (1991) has described fungi concerned in the

deterioration of cellulose dominated waste, but the authors main concerned was not of the fungi causing successional deterioration of cotton but was the microbial dynamisms as operating on the cotton fibre, being used in the spinning mill of Jhansi.

While comparing the microbial population of cotton obtained from site I, II & III it was observed that in site I the absolute numbers obtained in each sample was high to the ones obtained in the rest of the two sites. The reason was that at site I, the cotton fibres were comperatively shorter and were thoroghly mixed with dust and other organic waste. The cotton here remained lying for a considerably larger period. Thus flaring of organism must be due to the availability of large number of nutrient substances of various chemical constituents each having its own group of microorganism and thus the total number increased. When large number of organisms are acting on various substracts, they in addition of utilizing their substrates may also degrade a fair amount of other substance also. While on the other sites the cotton was raw and free from dust and other organic substances, hence had smaller number of organism's incorporation on it.

While comparing the percentage occurence of varius fungi at different site, it was observed that

in site I, twenty two sp. belongs to thirteen genera; Site II,eighteen sp. belonging to eleven genera; Site III,sixteen sp. belonging to nine genera; Site IV sixteen sp. belonging to nine genera ; Site V, twenty sp. belongiong to ten genra were isolated.

All togather twenty four sp. belonging to thirteen genera were isolated from the variuos sites studied. The monthly seasonal variation of differenct fungi on cotton samples has been showen in table XIII. A persual of table shows that **Fusarium solani** and **F. moniliforme** occured only in one sampling, among the other occasionally appearing sp. which appeared in 2-3 months were **Verticilium alboatrum**, **Paecilomyces variati** and **Aspergillus atropurpureus**. The monthly seasonal variation when compared with the indoor air to outdoor air it was observed, as found in table XIV and XV, that in the indoor air there were only ten species as compared to twenty sp. of the outdoor air. From the table it can be noticed that **A. niger**, **A. flavus**, **P. citrinum** and **Rhizopus nigricans** occured almost every month and were least effective by the seasonal variation with reference to the average number of forms in different seasons, it can be observed from table XVI that on an average number of form which occured on cotton during various months were as Nov., 7; Dec., 6; Jan., 6;, Feb., 10;, March-11; April-9; May-6; June-6;

TABLE - XIII

Seasonal variation in fungi from cotton samples of spinning mill

S.No.	Fungus	Months											
		NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
1-	Alternaria alternata	-	-	-	+	+	+	+	-	+	+	+	+
2-	Aspergillus flavus	+	+	+	+	+	+	+	+	+	+	+	+
3-	A. fumigatus	+	+	-	+	+	+	-	+	+	+	+	+
4-	A.nidulans	-	+	-	+	+	+	+	-	-	+	+	+
5-	A. niger	+	+	+	+	+	+	+	+	+	+	+	+
6-	A. oryzae	+	+	+	+	+	+	+	-	+	+	+	+
7-	A. sulphureus	+	+	+	-	+	+	+	+	+	+	+	+
8-	A. terreus	+	-	+	+	+	-	+	+	-	+	+	+
9-	A. ustus	-	+	+	+	+	+	+	+	+	+	+	+
10	Basidiobolus sp.	+	-	-	-	-	-	+	-	-	+	+	-
11-	Chaetomium globosum	+	-	+	-	-	-	-	+	-	+	+	-
12-	Cladosporium sp.	+	-	+	+	-	-	-	-	-	+	-	-
13-	Fusarium oxysporum	-	+	+	+	+	+	-	-	+	+	+	+
14-	F. solani	-	-	-	-	+	-	-	-	-	-	-	-
15-	Mucorhydrophilus	-	-	-	+	+	-	-	-	+	+	+	-
16-	Paecilomyces varioti	-	-	-	-	+	-	-	-	+	-	+	-
17-	Penicillium citrinum	+	+	+	+	+	+	+	+	+	+	+	+
18-	Penicillum sp.	-	-	+	+	+	+	-	-	-	-	-	-
19-	Phycomyces sp.	-	-	+	+	+	+	+	+	+	+	+	+
20-	Rhizopus nigricans	+	+	+	+	+	+	+	+	+	+	+	+
21-	Trichoderma viridi	-	-	-	+	+	-	-	-	-	+	+	-
22-	Verticillum alboatrum	-	-	+	-	+	-	-	-	-	-	-	-

TABLE - XIV

Seasonal variation in fungi obtained from indoor air of spinning mill

S.No.	Fungus	Months											
		NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
1-	Alternaria alternata	–	–	+	–	+	+	–	–	+	–	–	–
2-	Aspergillus flavus	+	+	+	+	+	+	+	+	+	+	+	+
3-	A. fumigatus	–	–	+	–	+	+	–	–	+	–	+	–
4-	A.nidulans	–	–	–	+	+	+	+	–	–	+	–	+
5-	A. niger	+	+	+	+	+	+	+	+	+	+	+	+
6-	A. oryzae	–	+	+	+	+	+	–	–	+	–	+	+
7-	A. sulphureus	+	–	+	–	+	+	+	–	+	–	+	+
8-	A. terreus	+	–	–	–	+	+	–	–	–	+	–	+
9-	A. ustus	–	+	+	+	+	+	–	–	+	–	+	+
10-	Chaetomium globosum	–	–	–	+	+	–	–	–	–	+	–	+
11-	Fusarium oxysporum	–	–	+	+	+	+	–	–	–	–	–	–
12-	Penicillium citrinum	+	+	+	+	+	+	+	+	+	+	+	+
13-	Phycomyces sp.	–	–	–	+	+	–	–	–	–	–	–	–
14-	Rhizopus nigricans	+	+	+	+	+	+	+	+	+	+	+	+
15-	Trichocderma viridi	–	–	–	–	+	–	–	–	–	–	–	–
16-	Verticillum alboatrum	–	–	–	–	+	–	–	–	–	–	–	+

TABLE - XV

Seasonal variation in fungi obtained from out door air of spinning mill

S.No.	Fungus	Months											
		NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
1-	Alternaria alternata	-	-	-	-	+	+	+	-	-	+	-	-
2-	Aspergillus atroperpureus	-	-	-	-	+	+	-	+	-	-	-	-
3-	Aspergillus flavus	+	+	+	+	+	+	+	+	+	+	+	+
4-	A. fumigatus	-	-	-	+	+	+	+	-	-	+	-	+
5-	A.nidulans	+	-	+	-	-	+	-	+	-	-	+	-
6-	A. niger	+	+	+	+	+	+	+	+	+	+	+	+
7-	A. oryzae	-	-	-	+	-	+	+	-	+	-	-	+
8-	A. sulphureus	-	+	-	+	+	+	+	-	+	-	-	+
9-	A. terreus	-	-	-	+	-	+	+	-	-	-	+	-
10-	A. ustus	+	-	+	-	+	+	+	-	+	-	+	-
11-	Basidiobolus sp.	-	-	-	-	-	-	+	-	-	-	-	-
12-	Chaetomium globosum	-	-	+	-	+	+	+	-	-	-	+	-
13-	Cladosporium sp.	-	-	-	+	+	-	-	-	-	-	-	-
14-	Fusarium moniliforme	-	-	-	-	+	-	-	-	-	-	-	-
15-	F. oxysporum	-	-	+	+	+	+	-	+	-	+	-	+
16-	Penicillium citrinum	+	+	+	+	+	+	+	+	+	+	+	+
17-	Penicillum sp.	-	-	+	-	-	-	+	-	-	-	-	-
18-	Phycomyces sp.	-	-	-	-	+	-	+	-	-	-	-	-
19-	Rhizopus nigricans	-	+	+	+	+	+	+	-	+	+	+	+
20-	Trichocderma viridi	-	-	-	+	+	+	-	-	-	-	-	-

TABLE - XVI

Average number of fungi in different season & enviorment

(On cotton and air out door & indoor)

S.No.	Months	Average number of forms on cotton in different months	Average number of forms in indoor air	Average number of forms in outdoor air
1.	November	07.0	05.0	04.0
2.	December	06.0	06.0	05.0
3.	January	06.3	10.0	09.0
4.	February	10.2	10.0	11.0
5.	March	11.0	14.0	15.0
6.	April	09.6	14.0	14.0
7.	May	06.6	05.0	15.0
8.	June	06.3	04.0	07.0
9.	July	08.2	09.0	06.0
10.	August	11.1	07.0	07.0
11.	September	11.0	08.0	08.0
12.	October	09.1	11.0	08.0

TABLE - XVII

Percentage frequency of fungal test organism at different sites

S.No.	Fungus	Site I	Site II	Site III	Site IV	Site V	Cotton	In door	Out door
1-	Alternaria alternata	29.2	16.7	12.5	20.8	25	-	-	+
2-	A. atropurpureus	-	-	-	-	12.5	-	-	+
3-	A. flavus	100	100	87.5	100	95.8	+	+	+
4-	A. fumigatus	41.7	29.2	20.8	25	25	+	+	+
5-	A.nidulans	25	-	25	29	20.8	+	+	+
6-	A. niger	100	100	95.8	100	100	+	+	+
7-	A. oryzae	29.2	20.8	12.5	41.6	20.8	+	+	+
8-	A. sulphureus	45.8	45.8	25	45.8	45.8	+	+	+
9-	A. terreus	33.3	20.8	8.3	20.8	16.6	+	+	+
10	A. ustus	50	16.7	33.3	37.5	37.5	+	+	+
11-	Basidiobolus sp.	8.3	8.4	4.2	-	4.2	+	-	+
12-	Chaetomium globosum	8.3	12.5	8.3	16.6	25	+	+	+
13-	Cladosporium sp.	8.3	16.7	-	-	8.3	+	-	+
14-	Fasarium moniliforme	-	-	-	-	4.2	-	-	+
15-	F. oxysporum	33.3	12.5	20.8	16.6	29.1	+	+	+
16-	F. solani	4.2	-	-	-	-	+	-	-
17-	Mucorhydrophilus	12.5	4.2	4.2	-	-	+	-	-
18-	Pacilomyces varioti	12.5	-	-	-	-	+	-	-
19-	Penicillium citrinum	91.7	71.4	70.8	75	70.8	+	+	+
20-	Penicillum sp.	7	12.5	-	-	8.3	+	-	+
21-	Phycomyces sp.	33.3	16.7	8.3	8.3	12.5	+	+	+
22-	Rhizopus nigricans	95.8	87.5	66.7	70.8	58.3	+	+	+
23-	Trichoderma viridi	25	-	-	4	12.5	+	+	+
24-	Verticillum alboatrum	4.2	4.2	-	4	9.1	+	+	+

July-8; August-11; September-11; & October-9. These numbers varried when isolations were done from air, inside the mill and out side the mill. Generally the number of forms out side the mill were comparatively larger as compared to the forms inside the mill. This variation might be due to the larger climatic variations and larger component of ecosystem involved in the outer atmosphere as compared to inner atmosphere. The percentage frequency of organisms which appeared at various sites are given in the table XVII. A perusal of table shows that A. niger, was in highest percentage, followed by A. flavus, Penicillium, citrinum and Rhizopus nigricans because of their occurence in highest percentage frequency, these organisrms were selected for further studies.

In all sites the author found that the absolute number of microorganism reached a peak level, two or three times alternatively followed by decline. This might be due to the type of organic matter being consumed. Initial rise shows the consumption of matter which represent the primary moulds.The secondry moulds concide with the second peak level and the third peak representing the tertiary moulds which are less and more stable. The author however could not distinguished between primary and secondry moulds as recorded by Kaarik (1974): Garrett (1951) & Bharat Rai (1970).

Numbering in definite sequence in strict sence could not be traced in successional pattern. This observation resembles with those of Mehrotra and Aneja (1974) and Kamal and Shrivastava (1975).

The percentage occurance of various fungi isolated from various sites also showed almost the same fungal organism in higher percentage occurance. Some species made occasional appearance. This might be due to the poor colonization ability of these organisms or due to the poor amount of enzyme secretion concerned in the process.

The on set of moisture leads to the burst of microbial activity **Aspergilli** were more abundant during the entire period of isolation, followed by **Rhizopus** and **Penicillium sp.** The fungi imperfecti occured on various occasions. **Aspergillus**, **Penicillium** and **Rhizopus** made up the major part of the population as observed by the author, similar observations were those of Pugh (1958). Many species multiply rapidly at first, dwindles as the envirnment changes. Temperature changes unavailability of food supply probably exerts the greater influence in determining the species of organisms comeprising the population at any one time.

SUB-SECTION - B

In the sub-section `A' above four fungal organisms i.e. Aspergillus niger, A. flavus, Penicillium citrinum and Rhizopus nigrican were found to be having the highest percentage frequency and therefore these were selected for further study on cellulolytic ability in this sub section.

The ability to utilize cellulose is an essential phenomena for saprophytic fungi, Melin (1948). This study has attracted many workers like Reese (1947); Siu (1951) Reese and Lavinson (1952). Siu has incuded Clodosporium herbarium and Alternaria tennius in a list of cellulolytic fungi isolated from competitive condition on cotton fabric. Garrett (1963) made a comparative study of cellulose decomposing ability in five fungi causing foot rot. Garrett (1966) has employed cellulolysis adequecy index as a measure of cellulose decomposing ability. The author used the method adopted by Garrett and the medium of Hogg to study the cellulose decomposing ability of the above four fungus and recorded the data in the table XVIII. Among these four A. niger was found to be most active resulting in 22% loss in dry weight of cotton, this was followed by A. flavus with 12.6%, P. citrinum 10.8% and Rhizopus nigricans with 11% loss in dry weight of cotton. These results point to the fact that these organisms posses good cellulolytic ability.

SUB SECTION - C

After determining cellulolytic ability of four selected fungal organism their competitive saprophytic colonization was studied in this section. The competitive saprophytic ability of these organisms were tested against the organisms which occur in the wash water suspension developed from cotton samples. The method followed was that of Wastie (1961). The wash water used was of two different sites. The data on radial growth are plotted separetely for 0 hrs., 24 hrs.and control. The graph obtained are present in the figures 13, 14, 15, 16 and data recorded in the table XIX. The observed results show that these organisms were quite successful colonizers. The cotton samples obtained from absorbing channel and that obtaioned from store gave almost the same results . The inhibitory effect as observed from the degree of supression in angle shows that supression in angle of growth rate of the test fungi is more in a sample of site I. This might be due to the secretion of more inhibitory substance produced from micro-organisms underlying the cellophane, by the organism of the site I. In case of all test fungi the growth was reduced when sample water suspension was inoculated and incubated for 24 hrs. before inoculating the test organism. It was also observed that test organisms were successful colonizers in the begining but after 24 hrs.

effective amount of inhibitory substances were produced which resulted in the depression in the growth rate. Thus there is a direct correlation between degree of supression of growth rate and the interval of inoculation between sample and test fungus.

SECTION III

CONTROL MEASURES

CHEPTER 14

INTRODUCTION

Fungicides & antibiotics are widely used to control the growth of various disease producing microorganism. These substances are agents that kills or inhibit microbial growth & development (Mehrotra, 1980), but are neither used in controling microbial spoilage of cotton fibre during its storage nor during spinning process. These substances may cause problems to the humans & thus unsafe to be used indiscriminataly & frequently at such places, where large number of mill workers are involved. The use of fungicides & antibiotics exert a positive or negative influence on the total microflora of the region. The pattern of microorganisms development after treatment can sometime cause unexpected problems, (Domsch (1964); Lockwood (1964);New hall(1955).). Unexpected benefits sometimes occur (Altman (1965); Collins (1965)).

There is an enormous amount of litrature available on fungicides, their chemical & physiological role. Among these " Principles of fungicide's action" by Horsfall (1956);"Fungicides" An advanced Treatise, in two volumes by Torgeson (1967, 1969);"Fungicidal chemistry" by Rich (1960); :Chemistry & Physiology of fungicidal action" by owns (1963); "Studies on nature of fungicides (Anon 1943)," Role of fungicides in modernizing agriculture in India"by Mehta

(1974); Chemical control of plant disease. An exciting future by Sbragia (1975).

The Adaptations of fungi to fungicides have been explained by Parry & wood (1958 , 1959); Jack & Smith (1952);Malati Majumdar & Som.D.(1987);Partridage & Rich (1962); Sridhar (1974); Shltala & Sinclair (1963) are few worth mentioning.

Inhibition of fungi is the underlying principle of almost all fungicides, they can effect its reproduction or growth . Effectiveness of fungicides depends upon quantity, nature & age of inoculum, as well as pH & concentration of medium (Sugunakar Reddy et. al., (1979);Donald E. Munnecke (1972).Apart from these factors Domsch (1964) however considered temperature & humidity among the enviromental factors to be important . The action of any fungicides is comparatively low at low temperature. When fungus growth it self is temperature dependent, only single fungicide or better fungicidal combination which are temperature dependent should be used. It is not unlikely that certain fungicides have different optimum temperature for different fungi. Humidity & temperature may effect both the penetration of chemicals & also degree of sensitivity of the fungus. Humidity must be maintened to acheive inhibitory effect of the fungicide.

Taking the investigation of some fungicides which are frequently used in agricultural (Thiram, Captan, Bavistin) & taking them with all the indispensable reservations, it is evident that normally when they are applied in normal recommended does they reduces the total number of fungi, bacteria significantly (Domsch 1964). Studies on the adaptation of fungi to fungicides has been carried out on <u>Penicillium notatum</u>, <u>P. roqueforti</u>, <u>Sclerotinia fructgena</u>, <u>Stenphylium sarcinaeforme</u>, <u>Botrytiscinetea</u> with fungicides like, $CuSo_4$, $Hgcl_2$, Glyodin, Captan, TMTD, Ziram, Nabam, Zineb, Thiram, Ferbam etc. In most of these cases Domsch (1964) found that the fungi develops tolerences to fungicides & retain that tolerence after transfer to fungicids free agar, In some cases the adopted strain shows a lower viability than the parent strain. Here Domsch 1964, is of the openion that strain with high fungicide tolerence but lower ability to competete with the parent strain will not survive in soil. Sharma & Chauhan (1985); Simbya <u>et. al.</u>, (1969); Sridhar (1974); Bad & Moss(1988); Abdel <u>et. al.</u>, (1981); Raynal (1973); Polyakav <u>et. al.</u>, (1963); Hausain <u>et. al.</u>, (1971) have studied the effect of certain fungicides against various fungi. Patil & Rao (1972) have studied the effect of fungicides on spore germination & mycelial growth of <u>Alternaria tenuis</u> & <u>Phyllastica gerbericola</u>.Fungicides

tested were Captan, Aureofungin, Brassicol & Thiram. Similarly Thirumalachar (1968): Rahanlkar & Neeragaard (1969) also studied the effect of fungicides on percentage inhibition of spore germination against several phytopathogenic fungi. Thakre & Johri (1973) have also studied the activity of Aureofungin on mycelial growth of some thermophilic fungi, in this study were included Aspergillus fumigatus &Rhizopus Sp. Thirumalachar et. al., (1964) have found that MIC of thermophilic fungi is much higher then that of mesophilic fungi.Parry & wood (1959) has given review on the adoptation of fungi to fungcides Thiram, Ziram, Zineb, Nabam, Ferbam & Captan. Singh et. al.,(1973) has used Aureofungin for inhibiting spore germination of 5 Alternaria sp.. This fungicide was also used by Cheema & Jeyarajan (1971), Sinha et. al., (1972), on Alternaria sp. The swelling phase of fungal spores under the influence of fungicides have been studied by Fletcher (1969); Adersan & Smith (1971). The swelling phase of germinating spores has been studied by Gattlieb & Tripathi (1968); Thakre & Johri (1973). Thakre & Johri while studing the swelling phase & spore germination of Aspergillus fumigatus and Rhizopus sp.have concluded that swelling phase is less sensitive to action of antibiotic than the subsequent germination phase.

Sharma & Chauhan (1985) have evaluated fungicides against four foliar cotton pathogen i.e. Alternaria, Helminthosporium, Curvularia & Myrothecium Simbya et. al., (1969) have worked on cotton boll rot in Arizona. The rot was found to be associated with Rhizopus arrhizus, Aspergillus niger, A. flavus, Penicillium sp., Fusarium roseum & Trichothecium & found that these were inhibited by Captan. Shridhar (1974) evaluated fungicides against Rhizopus sp.

The word "antibiotic" was first used by Waksman in 1942. According to Waksman 1944-47, "Antibiotic are antimicrobial agent of microbial origin that can inhibit the growth of bacteria or even destroy them & other micro-organism".

N.S. Egorov (1985) suggests the following defination for"antibiotic substances", antibiotics are specific product of metabolism or their modification with high physiological activity against individual group of micro-organism (Virus, bacteria, streptomyces fungi, algae,protozoa) or against malignant tumurs that can selectively slow down or completely inhibit their growth.

Antibiotics are not equally effective against all the different kind of microorganism. Some antibiotics are inhibitory to many different species

(broad spectrum). While some are inhibitory to only a few species of microorganisms. Johnson (1957); Mahendranath et. al., (1976); Gregory (1952) ; Chaursia et. al. (1973); Karzybsiki et. al., (1969); Lennette et. al., (1974); Thakre & Johri (1973), have studied the effect of certain antibiotics on various organisms.

Antibiotics may act by (1) effecting cell wall peptidoglycan biosynthesis (2) effecting cell membrane(3)Inhibiting DNA synthesis (4) Inhibiting protien synthesis, (Powar & Daginawala 1982). Before using any fungicides or antibiotics it is important to determine whether or not the said concentration is safe for humans and at the same time effective against the micro-organism. Thus experiment should be planned to study the proper concentration of the substance to be used.

Frankllin & Snow (1971, 1972); Pelezar et. al, (1977), Power & Daginawala (1982), have given good information on the biochemistry of antimicrobial agents. Allen (1968) while describing the mechanism of action of antibiotic has given M.I.C. of tetracyclin against **Streptococcus** & **Bacillus sp.** Jocoby & Gorini (1968) has described similarly the mechanism of action of streptomycin.Korzybski et. al., (1969) have discussed the origin, nature & properties of antibiotic. They discussed the role of Penicillin on

<u>Streptococcus</u> <u>sp.</u> & <u>Proteus</u> <u>sp.</u> Sensitivity of antibiotic has been tested by Mahendranth <u>et. al.</u>, (1976) against <u>Streptococcus</u> <u>sp.</u> and <u>Staphylococcus</u> <u>sp.</u> Effect of certain fungicides and antibiotics on spore germination of various fungi & bacteria have been studied by Patil & Rao (1972);Singh <u>et. al.</u>, (1973); Thakre & Johri (1973-74); Cheema & Jeyarajan (1971); Queshel <u>et.al.</u> , (1971). Effect of fungicides or antibiotic on mycelial growth of fungi have been studied by Thakre & Johri (1973); Agrawal & Rao (1974); Patil & Rao (1972).

Species & strain of species of microorganism have varying degree of susceptibility to different antibiotics. Further more, the susceptibility of an organism to given antibiotic may change especially during treatment. It is therefore important to know the identity of the microbe & the most satisfactory result in treatment, Michael, Chan & Krieg (1977). With the above consideration in this study, well known fungicides and antibiotics were screened against fungal and bacterial pathogen & saprophytic organisms which were more frequent in occurence during the period of isolations. The investigations were conducted on the following lines.

(1) Preliminary screening of fungicides & antibiotics, these were screened against the test micro- organism

with a view to select the fungicides/ antibiotics which could control the growth of the test organisms.

(2) Experiments were performed to find minimum inhibitory concentration of the selected fungicides & antibiotic.

(3) The fungicides and antibiotic which were found to be active against the test organism were further screened in different dilutions for their effect on the radial growth of th test organisms.

(4) The selected fungicides were tested for their effect on dry weight of the mycelial mat of the test organisms.

(5) The selected fungicides and antibiotics were tested for their effect on spore germination of the test organisms.

CHAPTER - 15

EXPERIMENTAL

Experiment A :- Preliminary screening of selected organisms against fungicides and antibiotics:

This study was based on the Principle of agar diffusion technique, where an inhibitory zone is developed if the test organism is susceptible to the diffusing substance.

Against the fungal organisms the fungicide & antibiotic used were Brassicol, Thiram, Captan, Bavistin, Actidone, Aureofungin, Streptomycin & Penicillin. Fungicides were tested in 5% concentration while antibiotic sutstances were tested in 25 ugm/ml concentration in Potato dextrose agar medium. Inhibitory zone obtained against the fungal organism i.e. **Aspergillus niger, A. flavus, Penicillium citrinum** & **Rhizopus nigricans** are given in table XX. From this preliminary screening experiment fungicide antibitic which gave promising results were selected and observed for their inhibitory effect in different concentrations Fungicides were tested in .020%, .010% & 0.005% concentrations while antibiotic in 15, 10, & 5 ug/ml concentrations. The inhibitory effects were recorded in table XXI.

Among the bacterial organisms **Staphylococcus aureus**, **Streptococcus pyogenes**, **Bacillus sp.**, **Proteus**

& **Mycobacterium tuberculosis** were selected for screening against Penicillin, Streptomycin, Kanamycin, Tetracyclin, Erythromycin, Rifamycin & Neomycin in 25 ugm/ml concentration on Nutrient agar / Lowenstein Jensen Medium. The tests were performed as per Karkaani (1969) paper disc method. Inhibitory zone obtained are given in the table XXII.

Prparation of Plates:- for **fungal organisms** sterilized plates were poured with PDA mixed with the above said concentrations of fungicide substance. When the agar solidified, previously sterilized glass cylinder of uniform volume & size were fixed asceptically along the periphery. These were kept uninoculated for 24 hrs. to let the surface film of water evaporate. The inoculation of the test organism was done with 0.7mm agar inoculation in the centre of the petriplate. Thereafter 24 hours of incubation at 28 degree C equal volumes (.2ml) of the above mentioned fungicides in desired dilutions were added to the marked cylinders. The time interval given inbetween the inoculation of the test organism & fungicides was to give sufficient time for the organisms to grew freely & the fungicide to act at the time when the fungus was activity growing. In case of Rhizpus which is the fast growing fungus the inoculation &inhibitory agents were added simultaneously for obvious reasons. Triplicates

inhibitory concentration (M.I.C.) so that it could be used when ever required in its minimum concentration, avoiding unneccesarily raised concentration that could

be harmfull for human dealing with cotton, if treated with said concentration of antibiotic or fungicides.

(I) <u>Minimum Inhibitory concentration of fungicides against fungal test organisms.</u>

In the above screening experiment Bavistin, Captan & Aureofungin were found to be promising hence selected for their study on M.I.C.

PDA was poured in petriplates with Bavistin & Captan in .020 , .010..005 % concentration. while Aureofungin in 10, 5 & 1 ugm/ml concentration. When the medium solidified the test fungus i.e. <u>Aspergillus niger</u>, <u>Aspergillus flavus</u>, <u>Penicillium citrinum</u>, <u>Rhizopus nigricans</u> were inoculated with 7 mm inocula discs in the centre of petriplates. M.I.C was determined by examing the growth of fungi in plates having fungicides in the above 3 dilutions. The lowest concentration in the series of dilution used having no growth after 48hrs. was taken as M.I.C of that fungicides. Result obtained in the experiment are given in table XXIII plates 1, 2, 3.

(II) <u>Minimum Inhibitory concentration of antibiotic against bacterial test organisms.</u>

By the tube dilution method described by Pelezar <u>et. al.</u>,(1977) one can determine the smallest amount of antibiotic required to inhibit the growth of organism in-vitro under the same principle increasing amount of antibiotic were placed in series of culture tube containing peptone broth. The concentration of antibiotic was marked on culture tubes. Peptone broth in culture tube with no antibiotic was run as control. Each series of tubes were then inoculated with a test organism incubated for 24hr at 32 degree C & the lowest concentration of antibiotic in the series which completely inhibited the growth of the test organism was noted & considered as M.I.C. of that antibiotic. For <u>Mycobacuerium tuberculosis</u> results obtained are given in the table after observing the growth for 6-8 weeks in Lowenstein & Jensen medium. For each antibiotic the series consisted of 15 dilutions i.e. .002, .005, .012, .025, .05, .075, .1,.15,.2,.3, .4, .5, .6, .9 & 1 Ugm/ml of the medium . Thus for each of the antibiotic used i.e. Pencillin, Streptomycin , Tetracyclin, Erythromycin & Rifamycin data were recorded for growth on all the above mentioned dilutions in the table XXIV A. The results observed are also shown in the plate

4, 5, 6. Only a few dilutions are shown in the plate for obvious reasons.In this experiment **Proteus sp** was found to be uninhibited by any of the antibiotic tested in above dilutions & **Mycobacterium tuberculosis** could not be inhibited in any of the above dilutions used by either Penicillim or Erythromycin . Thus in addition to the above dilutions a seperate series was prepared having 10, 15, 30, 45, 60, 75, 90, 100 & 200 ugm/ml of each antibiotic while for **Mycobacterium tuberculosis** only two series of dilution were prepared one for Penicillin & other for Erythromycin . Inoculation of the test organism & incubation was done as in the previous experiment & results are shown in table XXIV B.

Minimum inhibitory concentration obtained in the above experiments for the antibiotics tested with reference to the bacterial organisms are summaried in the table XXV.

EXEPRIMENT 'C':- Effect of selected fungicide / antibiotics on the radial growth of the test organisms.

From the above experiments fungicides / antibiotic which gave promising results were selected for their further study on the radial growth of the organisms. Bavistin Captan & Aurofungin were selected for their studies against **Aspergillus niger**, **A. flavus**, **Penicillium citrinum** & **Rhizopus nigricans.** Bavistin &

Captan were used in .020%, .010% & .005% concentrations while Aurofungin was used in 15 ugm/ml., 10 ugm/ml & 5 ugm/ml concentrations, the results obtained for their effect on the radial growth are given in table XXVI.

Streptomycin, Penicillin, Tetracyclin, Erythromycin & Rifamycin were found to be effective against <u>Staphylococcus aureus</u>, <u>Streptococeus pyogens</u>, <u>Bacillus sp.</u>, <u>Proteus sp.</u> & <u>Mycobecterium tuberculosis</u>. These were selected to study their effect on the radial growth of all the above mentioned bacterial organisms, except <u>Mycobacterium tuberculosis</u>. This organism is very slow so that primary cultures may not be macroscopically visible until 10 - 14 days after incubation or as late as 6 - 8 weeks.

These antibiotics were tested in three different concentrations. Concentrations selected for individual antibiotic were in relation to the result & observations made during the previous experiments on M.I.C.. These concentration & their observations are given in table XXVII.

<u>Preperation of plates</u> :- For this experiment fungicides / antibiotic were added in the above mentioned concentrations to PDA for fungi & to nutrient agar for bacteria at the time of pouriong & mixed by giving rotatory movement,when the medium of

plates were solidified then plates were inoculated with a 7 mm agar disc of the test organism in the centre. Control plates were kept without adding any fungicide or antibiotics. Triplicate were taken for each test. The discs were incubated at 28 degree C for fungi & 32 degree C for bacteria. The diameter of the growing colony was measured after every 24 hour upto 72 hour and the average of the triplicate were recorded in table XXVI & XXVII.

EXPERIMENT 'D':- Effect of fungicides on mycelial mat of test fungal organisms.

This experiment was performed to determine the effect of selected fungicides Aureofungin, Bavistin & Captan on the mycelial growth of Aspergillus niger, A. flavus, Penicillium citrinum, Rizopus nigricans in broth cultures. Fungi were grown on 35 ml. of potato dextrose broth medium in 100 ml. conical flask. The selected fungicides were mixed in desired dilutions, under sterlized condition before inoculating the flask, with 7 mm disc of test fungus. There different dilutions were used for each fungicides. Bavistin & Captan in .030%, .020%, .010% while Aureofungin in 10, 5 & 1 ugm/ml concentration. All flasks were incubated in triplicates for each organism. Control were also run where plain medium was used without incorporation of the fungicides.

After 10 days of incubation at 28 degree ± 2 degree C fungal mycelila mat from the flask were harvested from these still cultures, on previously weighed filtre paper. After oven drying at 80 degree C for 24 hr. net mycelial weight of each organism was calcultated. The data are shown in table XXVIII.

Bacterial test organisms were not considered for this experiment as they do not develop vegetative mycelium. The dry weight of their vegetative cell were negligible. The effect of antibiotic in broth on their population is considered in the next experiment.

EXPERIMENT 'E':- EFFECT OF SELECTED FUNGICIDES / ANTIBIOTIC ON SPORE GERMINATION OF TEST ORGANISMS.

The fungicides and antibiotic used during the above experiments were further screened for their effect on spore germination of test micro-organism. The dilution used were the same as used in the experiment on radial growth. Method of examination for fungi was different from that adopted for bacteria & is being discussed below. Experimental observations are recorded in the table XXIX & XXX.

(I) Influence of fungicides on spore germination of fungi :

Activily growing fungal test organisms *Aspergillus niger*, *A.flavus*, *Penicillium citrinum*,

Riozopus nigricans, were kept ready on PDA The spore germination was studied on agar discs (8 - 10 mm in diameter & 2 - 3 mm in thickness) of PDA Spore suspension of test fungus was prepared from fresh cultures of the above petriplates (app. 3 - 4 x 10 spores/ml) & a drop of this was placed on plain PDA agar discs which were placed in sterilised moist chamber & subsequently incubated at 28 degree C. These disc were run as control. Effect of Bavistin Captan & Aureofungin on spore germination of test organism were evaluated on agar discs which contained Bavistin & Captan in .005, .010 & .015% , concentration & Aurofungin in 5, 10 & 15 ugm/ml concentration. Germinated spores were counted microscopically after 4, 8 & 12 hrs. At least 100 spores were counted in each treatment Results are accorded in table XXIX.

(II) **Influence of antibiotic on spore germination of bacteria:**

Method adopted was that used by Queshel et. al.,(1971) with the modification that the bacterial spores were heated upto 60 degree C instead of 80 degree C. Spore suspension was prepared by growing the organism on nutrient broth (Oxoid). Plates were incubated at 32 degree C for about 40 hrs. & then harvested by washing of the growth with sterile, glass distilled water. This suspension was heated in

sterilized conical flask at 60 degree C for 15 minutes to kill vegetative cells, after which the spores were washed 3 times & resuspended in sterlized glass distilled water. This stock spore suspension was kept in the refrigerator ready for use & was used within 2 - 8 weeks of harvesting.

40 ml of spore suspension were taken in 250 ml. erlenmeyer flasks & kept in electric water bath. The flasks were placed in water at 20 degree C & then water was brought to 60 degree C. 1 ml of this spore suspension were then discharged in to 9 ml blank of sterile glass distilled water at room temperature & from these further 10 folds dilutions were prepared for plating in triplicates. The dilutions used was that which could develop 20 - 25 colonies in control plates.

Antibiotics was incorporated in nutrient agar at desired concentration. 0.3 ml volumes of the above spore suspension were spread on these soldified nutrient agar plates. These plates were than incubated at 32 degree C for 36 hrs.

Controls were also taken where no antibiotic was added to nutrient agar. The experiment was performed in triplicate & average number of colony per plate were counted after two days incubation at 32 degree C. The number of colonies developed after the

incubation were considered as the number of spores germinatedat 32 degree C. Results obtained are given in table XXX.

CHAPTER-16

RESULTS & CONCLUSION

In section II A the author isolated micro-organisms, colonizing cotton at an interval of 15 days for one year. During isolations the author found **Aspergillus niger**, **Aspergillus flavus**, **Penicillium citrinum** & **Rhizopus nigricans** to be most frequently occuring & dominating species. These organisms were then observed for their ability to decompose cotton in Sec. II "B". After confirming their ability to decompose cotton, these were then plated for their studies on competitive saprophytic colonization in Sec. II "C". From these observations it was clear that these organisms have significant ability to decompos cotton & were also active colonizers. During the isolation studies **Staphylococcus aureus**, **Streptococcus pyogenes**, **Bacillus sp.**, **Proteus sp.** & **Mycobacterium tuberculosis** were also frequently found. Since some of these are involoved in pathogenic human diseases, these organism together with the above four fungal organism were selected for their studies on preventive measure in this section. This was done with a view to find out the antibiotic or fungicides which could act as inhibitors for the growth & development of these organisms. The results obtained are being given below experiment wise.

EXPERIMENT A :- Preliminary screening of selected organisms against fungicides & antibiotics.

The results obtained in this experiment are being concluded for fungal organisms & Bacterial organisms separately.

Test against fungal organisms :-

Bioassay was done by agar diffusion technique. Fungal organisms selected from the previous section were *Aspergillus niger*, *A. flavus*, *Penicillium citrinum* & *Rhizopus nigricans*. These organisms were preliminrily screened against Brassicol, Thiram, Captan, Actidone, Bavistin, Aureofungin, Streptomycin & Penicillin. Fungicides used were Captan, Bavistin, & Aureofungin, gave promising results while none of the antibiotic could inhibit the fungal organisms. This will be evident from the perusal of the table XX. This experiment was simply a preliminary screening & aimed to select the fungicides or antibiotic which actively inhibited the growth of above mentioned fungal organism.

Results obtained in the table XX shows that Captan, Bavistin & Aureofungin gave promising activity thus these fungicides were selected for further screening in a separate test performed to test the fungicides in three different dilutions. Captan &

TABLE - XX

PRELIMINARY SENSTIVE TEST OF FUNGICIDES & ANTIBIOTIC AGAINST FUNGAL TEST ORGANISM

S. No.	ORGANISMS	FUNGICIDES - 0.5% CONCENTRATION					ANTIBIOTIC 25 ug/ml CONCENTRATI		
		ACTIDONE	BAVISTIN	BRASSICOL	CAPTAN	THIRAM	AUREOFUNGIN	PENICILLIN	STREPTOMYCIN
1	Aspergillus niger	+	++++	+	++++	++	++++	-	-
2	Aspergillus flavus	+	++++	+	++++	+	++++	-	-
3	Penicillium citrinum	-	++++	-	++++	+	++++	-	-
4	Rhizopus nigricans	-	-	-	++	-	+++	-	-

INHIBITORY ZONE GRADES :-

- = INHIBITION ABSENT
+ = 6 mm TO 8 mm
++ = 9 mm TO 11 mm
+++ = 12 mm TO 15 mm
++++ = 16 mm AND ABOVE

TABLE - XXI

Antifungal activity of the selected fungicides in three dilutions against fungal test organism -

(Clear Inhibitory zone in mm.)

S.No.	Organisms	Aureofungin ug ml			Bavistin			Captan		
		15	10	5	.020%	.010%	.005%	.020%	.010%	.005%
1.	Aspergillus niger	25	18	10	24	10	9.9	29	19	10
2.	Aspergillus flavus	26	20	12	26	12	9	30	24	102
3.	Penicillium citrinum	21	18	8	15	12	9	22	18	11
4.	Rhizopus nigricans	14	10	N	N	N	N	N	N	N

N = inhibition zone not developed

Bavisin were used in .020%, .010%, .005% concentration while Aureofungin was used in 5 ugm/ml, 10 ugm/ml & 15 ugm/ml concentration. The zone of inhibition as given in table XXI will show that **Rhizopus nigricans** gave inhibitory effect only against Aureofungin & the maximum inhibition of 14mm was observed when the fungicide was used in 15 ug/ml concentration.

With reference to other organisms Captan gave the best result against rest of 3 fungal test organism. **Aspergillus flavus** was inhibited the most, followed by **A. niger** & **Penicillium citrinum**, respectively. Among the said fungicides Captan was followed by Aureofungin & Bavisin successively with reference to the three above mentioned fungal organism.

Test against bacterial organism:- Here the test organisms used were **Staphylococcus aureus**, **Streptococcus pyogens**, **Bacillus sp.**, **Proteus sp**, **Mycobacterium tuberculosis** and antibiotics were used in 25 ug/ml concentration. The test was performed by using paper disk method. The antibiotics used were, Aureofungin, Erythromycin, Kannamycin, Neomycin, Penicillin, Rifamycin, Streptomycin & Tetracyclin. The results obtained in the table XXII show that **Proteus sp.** was not inhibited by any of the above antibiotics. Aureofungin on the other hand was ineffective against

TABLE - XXII

Preliminary sensivity test of antibiotic against bacterial test organism by filter paper dick of 25 ug/ml concentration

(Inhibitory zone in cm.)

Name of organisms	(1) Aureofungin	(2) Erythromycin	(3) Kannamycin	(4) Neomycin	(5) Penicillin	(6) Rifamycin	(7) Streptomycin	(8) Tetracyclin
Staphylococus aureus	0.00	1.30	1.60	0.70	2.60	1.90	0.90	1.80
Streptococcus pyogenes	0.00	1.90	1.00	0.60	2.80	1.80	1.10	2.10
Bacillus sp.	0.00	1.30	1.50	0.40	2.70	1.60	1.00	1.40
Proteus sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mycobacterium tuberculosis	0.00	0.90	0.70	0.50	0.50	1.80	1.90	0.60

all the test bacterial organisms. Remaining seven antibiiotic gave positive results against all the tests bacterial organisms except **Proteus sp.** as already stated above. **Pencillin** gave the best inhibitory effect on **S. aureus. S.Pyogenes** & **Bacillus sp.** followed by Rifamycin, Tetracyclin & Erythromycin, against **M. tuberculosis** Streptomycin gave the best activity followed by Rifamycin & Erthromycin.

EXPERIMENT 'B':- Determination of minimum inhibitory concentration of fungicides & antibiotics against the selected test organisms.

This experiment was also performed separately for fungal & bacterial organism, as such results are being given under separate headings.

M.I.C. of fungicides against fungal test organisms :-

Since the results obtained from experiment 'A', antibiotic could not give positive results thuse only fungicides were considered for determination M.I.C. concentration; Aureofungin. Bavistin & Captan which gave promising results in the experiment 'A' were used in this experiment against test organism, **Aspergillus niger**, **A. flavus**, **Penicillum citrinum,** & **Rhizopus nigricans**. Bavistin & Captan were used in .005%, .010% & .020% concentrations, while Aureofungin was used in 1 ugm/ml, 5 ugm/ml, & 10 ugm/ml. concentrations. M.I.C. was

TABLE - XXIII

MINIMUM INHIBITORY CONCENTRATION OF SELECTED FUNGICIDES AGAINST FUNGAL TEST ORGANISM

S. No.	ORGANISMS	FUNGICIDES & GROWTH GRADES									M.I.C.		
		AUREOFUNGIN (ug/ml)			BAVISTIN (%)			CAPTAN			AUREOFUNGIN (ug/ml)	BAVISTIN (%)	CAPTAN (%)
		10	5	1	0.020	0.010	0.005	0.020	0.010	0.005			
1	Aspergillus niger	-	++	+++	-	-	++	-	-	++	10	.010	.010
2	Aspergillus flavus	-	++	+++	-	-	++	-	-	+++	10	.010	.010
3	Penicillium citrinum	-	++	+++	-	-	++	-	-	++	10	.010	.010
4	Rhizopus nigricans	++	+++	++++	++++	++++	++++	+++	+++	++++	10-15	N	N

determined by examining the growth of fungi in plates having fungicides in the above dilutions. The Lowest concentration in the series of dilution used having no growth after 48 hrs. was taken as M.I.C. of that fungicides. The results obtained in the table XXIII & plates 1, 2 & 3 shows that Bavistin & Captan had no growth at .010% concentration & .020% concentration of Aspergillus niger, A. flavus & Penicillium citrinun. Since the .010% was the lower concentration hence considered as M.I.C. of captan & Bavistin against the above mentioned 3 fungal organisms. Aureofungin also completely inhibited the growth of these organism at 10 ugm/ml. Thus M.I.C. of Aureofungin will be 10 ug/ml concentration Rhizopus nigricans was not inhibited by either Bavistin or captan, however a moderate growth was obtained when Aureofungin was used in 10 ugm/ml concentration thus it was assumed that its M.I.C. would be between 10 to 15 ugm/ml concentration.

M.I.C.of antibiotic against bacterial test organisms:-

This experiment was performed according to the method discribed by Pelezar et. al., (1977).

Increasing dilution series of the antibiotic were prepared in peptone broth. For each antibiotic tested a series of 15 dilutions were used. The lowest

concentration of antibiotic which completly inhibited the growth of test organism was considered as M.I.C. for that organism.

Results obtained of this experiment as given the table XXIV "A" shows that Penicillin & Rifamycin were M.I.C. at vary low dilutions against <u>Staphylococcus aureus</u>, <u>Streptococcus pyogenes</u> & <u>Bacillus sp.</u> these were followed by Tetracyclin, Erythromycin & Streptomycin respectively. <u>Mycobacterium tuberculosis</u> was inhibited completly by Rifamycin at .05 ugm/ml & Streptomycin at .5 ugm/ml concentration, thus these are M.I.C. of the said antibiotic. The results obtained are shown in the plate No. 4,5 & 6.Only few of these dilutions are shown in this plate for obvious reasons.

Since <u>Proteus sp.</u> shown in plates could not be completly inhibited by any of these antibiotics in the above dilutions tested & <u>Mycobactirum tuberculosis</u> could not be inhibited by Penicillin & Erythromycin thus these antibiotics were tested again in higher concentration i.e. in 10, 15, 30, 45, 60, 70, 90, 100 & 200 ugm/ml concentration.

The result obtained as shown in a taboe XXIV "B" clearly establish the M.I.C. concentration of Rifamycin at 30 ugm/ml, Erythromycin at 90 ugm/ml

TABLE - XXIV A

Experimental results obtained during the study for M.I.C. of antibiotic against bacterial test organism .

Concentration of antibiotic in (ug/ml)

S.NO.	Antibiotic	Organism	0.002	0.005	0.012	0.025	0.05	0.075	0.1	0.15	0.2	0.3	0.4	0.5	0.6	0.9	1.0
1.	Streptomycin	Staphylococcus aureus	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-
		Streptococcus pyogenes	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-
		Bacillus sp.	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-
		Proteus sp.	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Mycobacterium tuberculosis	-	-	+	+	+	+	-	+	+	+	+	-	-	-	-
2.	Penicillin	Staphylococcus aureus	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
		Streptococcus pyogenes	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-
		Bacillus sp.	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
		Proteus sp.	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+
		Mycobacterium tuberculosis	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
3.	Erythromycin	Staphylococcus aureus	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-
		Streptococcus pyogenes	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
		Bacillus sp.	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-
		Proteus sp.	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Mycobacterium tuberculosis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Contd. ....

S.NO.	Antibiotic	Organism	0.002	0.005	0.012	0.025	0.05	0.075	0.1	0.15	0.2	0.3	0.4	0.5	0.6	0.9	1.0
4.	Tetracyclin	Staphylococcus aureus	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-
		Streptococcus pyogenes	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
		Bacillus sp.	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
		Proteus sp.	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Mycobacterium tuberculosis	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5.	Rifamycin	Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Streptococcus pyogenes	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
		Bacillus sp.	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-
		Proteus sp.	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
		Mycobacterium tuberculosis	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

TABLE - XXIV B

Experimental observations on raised antibiotic concentration to obtain M.I.C. against Proteus & Mycobacterium tuberculosis

(Concentration of antibiotic in ug/ml)

S.NO.	Organisms	Antibiotic	10	15	30	45	60	75	90	100	200
1.	Proteus sp.	Streptomycin	+	+	+	+	+	+	+	-	-
		Penicillin	+	+	+	+	+	+	+	+	-
		Erythromycin	+	-	+	+	+	+	-	-	-
		Tetracyclin	+	+	+	+	+	+	+	-	-
		Rifamycin	+	+	-	-	-	-	-	-	-
2.	Mycobacterium tuberculosis	Penicillin	+	-	-	-	-	-	-	-	-
		Erythromycin	-	-	-	-	-	-	-	-	-

TABLE - XXV

MINIMUM INHIBITIRY CONCENTRATION OF FIVE ANTIBIOTICS AGAINST 5 SELECTED BACTERIA

ORGANISM	ERYTHROMYCIN (ug/ml)	PENICILLIN (ug/ml)	RIFAMYCIN (ug/ml)	STREPTOMYCIN (ug/ml)	TETRACYCLIN (ug/ml)
Streptococcus aureus	0.60	0.02	0.005	0.90	0.1
Streptococcus pyogenes	0.05	0.10	0.05	0.90	0.05
Baccillus sp.	0.40	0.02	0.070	0.60	0.1
Proteus sp.	90.00	200.00	30.00	100.00	100
Mycobacterium tuberculosis	10.00	15.00	0.05	0.50	N

N - Not observed.

concentration, Tetracyclin & Streptomycin at 100 ugm/ml concentration, while Penicillin at 200 ugm/ml concentration against <u>Proteus sp.</u>. Exept Rifamycin the rest of the antibiotic inhibited <u>Proteus sp.</u> at a very high concentrations theretore it could be regarded as resistant to these antibiotics. Penicillin & Erythromycin gave M.I.C. at 15 ugm/ml & 10 ugm/ml concentration respectively against <u>Mycobaterium tuberculosis</u>. M.I.C. obtained in these experiments with refrence to bacterial organisms are summarised in table XXV.

<u>EXPERIMENT 'C':- Effect of selected fungicides & antibiotics on the radial growth of the test organisms</u>.

As described in the above experiment, the results for fungi & bacteria will be concluded here also separately for their studies on the radial growth of test organism.

Bavistin & Captan were used in concentration of .020%, .010% & 005%, while Aureofungin was used 15, 10 and 5 ugm/ml concentration. Fungal organisms, used here were same as above. The observations recorded as given in table XXVI confirm theprevious observations on sensitivity & antifungal activity of the fungicides. Three fungicides Captan, Aureofungin &

TABLE - XXVI

Effect of fungicides on the radial growth of fungal test organism

Fungicides/antibiotic radial growth in cm.

S.No.	Organism	No. of day after inoculum	Bavistin			Captan			Aureofungin			Control
			.020%	.010%	.005%	.020%	.010%	.005%	15ug/ml	10ug/ml	5ug/ml	
1.	Aspergillus niger	Initial	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
		1	0.7	0.7	0.9	0.7	0.7	1.0	0.7	0.7	0.9	1.4
		2	0.7	0.7	2.5	0.7	0.7	1.6	0.7	0.7	1.5	3.2
		3	0.7	0.7	3.4	0.7	0.7	3.0	0.7	0.8	2.7	4.1
		4	0.7	0.8	4.2	0.7	0.7	4.0	0.7	0.9	3.2	4.6
2.	Aspergillus flavus	Initial	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
		1	0.7	0.7	1.0	0.7	0.7	1.0	0.7	0.7	0.9	1.1
		2	0.7	0.7	1.8	0.7	0.7	1.6	0.7	0.7	1.4	3.0
		3	0.7	0.7	2.6	0.7	0.7	2.5	0.7	0.7	2.8	3.8
		4	0.7	0.7	3.5	0.7	0.7	3.1	0.7	0.8	3.0	4.7
3.	Penicillium citrinum	Initial	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
		1	0.7	0.7	0.8	0.7	0.7	0.9	0.7	0.7	0.8	0.9
		2	0.7	0.7	1.2	0.7	0.7	1.1	0.7	0.7	1.0	1.2
		3	0.7	0.7	1.6	0.7	0.7	1.3	0.7	0.7	1.4	2.5
		4	0.7	0.7	2.5	0.9	0.7	2.4	0.7	0.7	2.0	3.8
4.	Rhizopus nigricans	Initial	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
		1	2.0	2.2	2.0	2.2	2.0	2.3	0.7	0.9	2.0	2.6
		2	4.5	5.2	5.6	5.0	5.2	5.3	1.0	1.5	2.6	4.5
		3	full	full	full	full	full	full	1.2	1.9	3.2	full
		4	full	full	full	full	full	full	1.5	2.3	4.4	full

Bavistin exhibited significant control on the radial growth. Again **Rhizopus** manage to grow unhibited in Captan & Bavistin however slight inhibitory effects were observed in Aureofungin at all concentrations used, maximum inhibition was observed at 15 ugm/ml concentration. The growth obtained here was negligible as compared to that of control. The growth in 10 ugm/ml was also very slow, measuriong 2.3 mm after 4 days. For the rest of the organism the inhibitory effect was in the same pattern i.e. by Captan followed by Aureofungin & Bavistin.

In all the three fungicides used against three fungal organisms i.e. **Aspergillus niger**, **A. flavus** & **Penicillium citrinum**, though slight growth was recorded at the lowest concentration used but was inhibited at the two above concentration.

Against the bacterial test organisms antibiotic used were Erythromycin, Penicillin Rifamycin, Streptomycin & Tetracyclin. While studying their effect on the radial growth and recording their data it was observed that even in the control **Mycobacterium tuberculosis** was very slow growing organism so that the primary culture does not become macroscopically visible until 10 - 14 days of incubation or as late as 6 - 8 weeks, thus this

TABLE - XXVII

Effect of antibiotics on radial growth of bacterial test oranisms.

Antibiotic in ug/ml concentration & radial growth in cm.

Organisms	Hrs. ofter incubation	Control	Erythromycin			Penicillin			Rifamycin			Streptomycin			Tetracyclin		
			.6	.2	.05	.05	.025	.012	.05	.025	.005	2.4	1.2	.6	.1	.05	.025
Staphylo- coccus aureus	0	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7
	24	.9	.7	.8	.9	.7	.7	.8	.7	.7	.7	.7	.7	.8	.7	.9	.9
	48	1.5	.7	.9	1.2	.7	.7	1.0	.7	.7	.7	.7	.7	1.2	.7	1.0	1.1
	72	2.0	.7	1.3	1.6	.7	.7	1.1	.7	.7	.7	.7	.7	1.6	.7	1.1	1.3
Strepto- cocus pyogenes	0	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7
	24	.8	.7	.7	.7	.7	.8	.8	.7	.8	.9	.7	.7	.8	.7	.7	.9
	48	1.2	.7	.7	.7	.8	.9	1.0	.7	1.0	1.1	.7	.7	1.0	.7	.7	1.0
	72	1.5	.7	.7	.7	1.0	1.0	1.2	.7	1.1	1.2	.7	.7	1.0	.7	.7	1.2
Bacillus sp.	0	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7
	24	1.1	.7	.8	.9	.7	.7	.8	.9	1.0	1.0	.7	.7	.7	.7	.8	.9
	48	2.4	.7	1.2	1.4	.7	.7	1.0	2.0	2.0	2.2	.7	.7	.8	.7	.9	1.4
	72	2.9	.7	1.6	2.5	.7	.7	1.3	2.2	2.6	2.7	.7	.7	.8	.7	1.2	1.6
Proteus sp.	0	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7
	24	2.0	2.0	1.8	1.9	1.8	1.9	1.8	2.0	2.1	2.0	1.6	1.8	1.7	1.9	1.8	2.0
	48	4.0	3.7	3.9	3.8	3.9	3.7	4.0	3.7	3.8	3.9	3.6	3.8	3.9	3.6	3.9	4.0
	72	5.8	5.6	5.8	5.8	5.6	5.5	5.6	5.6	5.7	5.8	5.7	5.7	5.8	5.7	5.8	5.9

organism could not be studied for its affect on radial growth.

The rest of the bacterial organisms were the same as taken in the previous experiment. The concentrations used in this experiment were in relation to results & observations made during the experiment for M.I.C.. The result recorded as given in table XXVII shows that all the five antibiotics gave good inhibitory effect on all bacteria except the **Proteus sp.** which grew almost uneffected. **Bacillus sp.** was not inhibited by Rifamycin at the concentration used in this experiment. These observation once again confirmed observations recorded in the previous experiments.

EXPERIMENT 'D':- Effect of fungicides on the mycelial growth of fungal test organisms.

The experiment was conducted against fungal test organisms only, since the bacteria do not develop vegetative mycelium and dry weight of the vegetative cells were negligible. The effect of these antibiotics on their number is considered in the nest expriment on spore germination.

Fungicides as well as fungal organisms used were the same as selected for the previous experiments. The experiment as conducted in broth culture under

TABLE - XXVIII

Effect of fungicides on mycelial mat (dry weight basis) of fungal test organisms.

(Dry weight of fungal mycelium in mg.).

S. No.	ORGANISMS	BAVISTIN .030%	.020%	.010%	CAPTAN .030%	.020%	.010%	AUREOFUNGIN ugm/ml 10	5	1	CONTROL
1.	Aspergillus niger.	N	10	25	N	N	N	N	190	218	220
2.	Aspergillus flavus.	N	5	20	N	N	N	N	64	120	128
3.	Penicillium citrinum.	N	7	23	N	N	N	N	72	182	190
4.	Rhizopus nigricans.	115	119	125	110	117	119	25	116	121	130

N - No fungal growth.

three different dilutions. Results obtained are given in the table XXVIII. The dilutions used for Bavistin & Captan was .030, .020, .010% While for Aureofungin was 10, 5 & 1 ugm/ml concentration. As recorded in the above table the mycelial mat in broth culture shows that the growth was very less as compared to that of control except in case of **Rhizopus nigricans**. **Rhizopus nigricans** gave least mycelial growth i.e. 25 mg. under the influence of 10 ugm/ml concentration of Aureofungin this was compared to 130 mg of control. The rest of the fungal organism behaved differently in different fungicides. Captan completly inhibited the growth all the three fungal organisms, in all the above three concentrations used. On Bavistin growth was recorded at .020% & .10% concentration, this growth is quite negligible as compared to that of control. In Aureofungin also growth were recorded at 1 ugm/ml & 5 ugm/ml concentration. The growth was considerably decrease as the concentration was increased to 5 ugm/ml against **Aspergillus niger**, **A. flavus** & **Penicillium citrinum** but **Rhizopus nigricans** was almost uneffected. Its dry weight was 116 mg as compared to 130 mg of the control. When the concentration of this fungicide was increased to 10 ugm/ml concentration the growth was completely inhibited of **A. niger**, **A. flavus** and **P. citrinum**, **Rhizopus nigricans** developed 25 mg dry weight of mycelium as compared to 130 mg in control. From the

over all study it can be concluded that Captan complctly inhibited the growth of the three organism <u>A. niger</u>, <u>A. flavus</u> & <u>P. citrinum</u> in all the concentrations used. Aureofungin and Bavistin completly inhibited the growth of above three organism when used in concetration of 10 ugm/ml and .030% respectively. These two fungicides showed growth in the two lower concentration used but the two in above concentrations considerably reduced the growth. The growth obtained in Bavistin was negligible as compared to that of control. <u>Rhizopus nigricans</u> were uneffective except in 10 ugm/ml concentration of Aureofungin.

<u>EXPERIMENT 'E'</u> :- <u>Effect of selected fungicides and antibiotics on spore germination of test organisms</u>.

During studies on the effect of fungicides and antibiotics on spore germination of test micro-organism the dilution used were the same as used in the experiment of radial growth. Experimental results obtained are being concluded below separately for fungal & bacterial organisms.

<u>Influence of fungicides on spore germination of fungal organisms</u> :-

Captan, Bavistin & Aureofungin were subjected to their study on spore germination under the

microscope on agar disc of PDA against the four above fungal organisms Captan & Bavistin were used in .005%, .010%, & .020% concentration while Aureofungin was used in 1, 5,10 and 15 ugm/ml. The results as recorded in the table XXIX shows that Captan & Aureofungin gave better results as compared to Bavistin again. **Rhizopus nigrican** again showed an inhibitory effect completely different from other three fungal organisms. In the earlier experiments it was though observed that the radial mycelial growth was almost uneffected by these fungicides but in this experiment inhibitory effect was noted against all fungicides on spore geremination. Aureofungin gave 34% spore germination as compare to 100% after 12 hrs. in that of control with in the same duration Captan gave 40% and 50% spore germination at .020% & .010% concentration respectively. This was 100% in Bavistin in both these concentrations. In presence of all fungicides as the concentrations was increased, spore germination of **Rhizopus nigricans** was also reduced. In **Aspergillus niger**, **A. flavus** & **Penicillium citrinum** no spore germination was recorded at .020% concentration of either Captan or Bavistin and 10 & 15 ugm/ml concentration of Aureofungin. At .010% concentration Captan completely inhibited spore germination of all the three test organisms. While in Bavistin **A. flavus** manage to germinate a very few spores i.e. 7% after 12 hrs. interval. This number is

TABLE - XXIX

Percentage spore Germination of fungal organism in presence & absence of fungicides.

S. No.	Organism	Time (hrs.)	Control	Bavistin .005%	Bavistin .010%	Bavistin .020%	Captan .005%	Captan .010%	Captan .020%	Avreofungin (ug/ml) 1	Avreofungin (ug/ml) 5	Avreofungin (ug/ml) 10	Avreofungin (ug/ml) 15
1.	Aspergillus niger	4	S(100)	S(70)	N	N	S(75)	N	N	S(70)	S(75)	S(5)	N
		8	90	65	S(5)	N	70	S(10)	N	68	60	S(7))	N
		12	100	70	S(15)	S(5)	75	S(5)	S(5)	100	68	S(10)	S(2)
2.	Aspergillus flavus	4	S(100)	S(88)	S(8)	N	S(90)	N	N	S(70)	S(40)	N	N
		8	8	68	5	N	70	N	N	66	36	N	N
		12	100	82	7	S(5)	85	S(5)	N	100	72	S(10)	N
3.	Penicillium citrinum	4	N	N	N	N	N	N	N	N	N	N	N
		8	N	N	N	N	N	N	N	N	N	N	N
		12	S(100)	S(80)	S(60)	N	S(75)	S(5)	N	S(100)	S(90)	N	N
		24	100	70	6	N	65	S(10)	N	100	88	S(5)	N
4.	Rhizopus nigricans	4	S(100)	S(100)	S(80)	S(70)	S(50)	S(30)	S(35)	S(100)	S(100)	S(70)	S(40)
		8	100	100	70	68	48	S(40)	S(32)	100	98	70	30
		12	100	100	100	100	60	50	40	100	100	90	34

N - No germination of spores

S( ) - Swollen spore

again negligible as compared to 100% germination of the control. <u>P. citrinum</u> at this concentration and interwal showed 10% swelling of spore, however after 24 hrs. interval 6% spore manage to germinate, this is again very less as compared to 100% germination of the control. At .005% concentration in both Captan & Bavistin these fungal organisms had 60% to 80% germination however when the concentration were increased to 0.010% concentration the percentage germination reduced considerably. Aureofungin considerably reduced the percentage germination in 5 ugm/ml concentration as compare to the one when it was used in 1 ugm/ml. Aureofungin was most active against <u>P. citrinum</u> followed by <u>A. flavus</u> & <u>A. niger</u>.

<u>P. citrinum</u> was slow in spore germination even in control experiment germination took place only after 24 hr.

<u>Influence of antibiotics on spore germination of bacterial organisms</u>:-

This experiment was conducted on the same bacterial organisms, antibiotic & dilution as used during the experiment of radial growth method followed was that of Queshel <u>et. al.</u> 1971. In this number of colonies develop after incubation were consiodered as the number of spore germinated. The results given the

TABLE - XXX

Effect of antibiotics on spore germination of test bacteria.

Concentration of antibiotics in ugm/ml & numbers of colonies develop (average)

(Results are given in average number obtained perplate / % germination in relation to control).

S. No.	ORGANISMS	CONTROL	ERYTHROMYCIN .6	ERYTHROMYCIN .2	ERYTHROMYCIN .05	PENICILLIN .05	PENICILLIN .025	PENICILLIN .012	RIFAMYCIN .05	RIFAMYCIN .025	RIFAMYCIN .005	STREPTOMYCIN 2.4	STREPTOMYCIN 1.2	STREPTOMYCIN .6	TETRACYCLIN .1	TETRACYCLIN .05	TETRACYCLIN .025
1.	Streptococus pyogenes.	18	N / 0%	N / 0%	1 / 5.5%	12 / 5.5%	16 / 88.8%	17 / 94%	N / 0%	15 / 83%	16 / 88.8%	N / 0%	N / 0%	9 / 50%	N / 0%	N / 0%	16 / 88.8%
2.	Staphylococcus aureus.	22	N / 0%	9 / 40%	20 / 90%	N / 0%	N / 0%	8 / 36.3%	N / 0%	N / 0%	N / 0%	N / 0%	N / 0%	18 / 81.8%	N / 0%	8 / 36.3%	21 / 95.4%
3.	Bacillus sp.	24	N / 0%	12 / 50%	23 / 95.8%	N / 0%	N / 0%	14 / 58.4%	7 / 29.1%	18 / 75%	23 / 95.5%	N / 0%	N / 0%	1 / 41%	N / 0%	12 / 50%	22 / 91.6%
4.	Proteus sp.	Abundent Spore Germination	Abundent Spore Germination			Abundent Spore Germination			Abundent Spore Germination			Abundent Spore Germination			Abundent Spore Germination		

N = No colony developed

table XXX confirm the previous observations on the inhibitory effect of the bacterial organisms. Here also <u>**Mycobacterium tuberculosis**</u> was not considered on the number of spore germinated. Against <u>**Streptococcus pyogenes**</u>, Erythromycin, Penicillin, Rifamycin & Tetracycline when used in .05 ugm/ml concentration gave almost the same result. Only one colony developed in each Erythromycin & Penicilin & no colony developed in Rifamycin & Tetracyclin. The result were almost the same in dilution of .025 ugm/ml concentration. In Streptomycin complete inhibition was recorded at a higher concentration i.e. 1.2 ugm/ml concentration.

Against <u>**Staphylococus aureus**</u>, Penicillin & Rifamycin completly inhibited the spore germination at 0.025 ugm/ml concentration while in Tetracycline & Erythromycin at .1 & .6 ugm/ml concentration respectively. In Streptomycin complete inhibition was recorded at higher concentration i.e. 1.2 ugm/ml. Against <u>**Bacillus sp.**</u>, spore germination was not inhibited completely at .05 ugm/ml concentration by Erythromycin, Rifamycin and Tetracyclin. Streptomycin developed only one colony at 0.6 ugm/ml concentration. While Penicillin resulted complete inhibition at .025 ugm/ml concentration. Erythromycin resulted complete inhibition at .6 ugm/ml concentration & Tetracyclin at

Proteus sp. remain uneffected by any of the antibiotic tested & the spore germination remain unaltered.

CHAPTER -17

SUMMARY AND DISCUSSION

During the experiments conducted in section II **Aspergillus niger**, **A. flavus**, **Pencillium citrinum** and **Rhizopus nigricans** were found to be of frequent occurrence, had good cotton decompositing ability and were also significant competitive saprophytic colonizers. Apart from plant diseases and decomposition these organism are well known for causing various diseases and complications in men & animals. **A. niger** is one of the frequent agent of otomycosis and pulmonary "fungus ball". **A. flavus** is a common apportunistic organism in patients with altered host defence. It is also responsible in causing pulmonary disease of captive and wild birds. Afflotoxins produceed by some strains of this species have direct toxicity & long term carcinogenic effect on animal tissue. Similarly species of **Penicillium** are well known for penicillosis and contamination of eczematoid lessions, open ulcers, urine, sputum etc. While **Rhizopus** often get involved in Mucormycosis (Chester W- Emmons et. al., (1977); Diener et. al., (1976) has proved the toxigenicity of **Aspergillus**, **Penicillum**, **Fusarium** and **Alternaria sp.** on rats and chicke embryos. Some stains of **Aspergillus flavus** has been found to be toxic (Rati & Ramalingam (1979); Rashmi Tewary (1991) have also reffered to the allergic responses of **A.**

niger, A. flavus, Rhizopus nigricans and Penicillium citrinum. Diener et. al., (1976) has justified continueing research on toxic metabolites to overcome hazards to human health that would result from such organisms.

During the isolation studies Bacillus sp., Proteus sp., Staphylococcus aureus, Streptococcus pyogenes & Mycobacterium tuberculosis were also frequently found, some of these are not inhabitant of cotton but might have come directly through droplet nuclei caused by coughing, sneezing & conversation or indirectly by fomites used by infected persons.

Since some of these organism are involved in pathogenic diseases of men & animal, these organisms together with the above four fungal organisms were selected for their studies on preventive measures in this section.

Though fungicides have played a significant role in agricultural economy of India, particularly in ensuring the health of plantation and commercial crops but so far no work of any significance has been undertaken for their use in preservation of textile raw material like cotton and prevention of organisms involved in allergies and pathogenesis. Thus well known fungicides and antibiotics were explosed in this section for their

inhibitory effect on the growth and development of these organisms . The experiment performed and result obtained in this section are being summerised and discussed below experiment-wise.

EXPERIMENT 'A':- Preliminary screening of selected organisms against fungicides & antibiotics.

1. **Test against fungal organisms :-**

The study was conducted following the principle of agar diffusion technique and observation was made for the development of inhibition zone by succeptable organism. *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum* & *Rhizopus nigricans*, were screened against Actidone, Bavistin, Brassicol, Captan, Thiram, Aureofungin, Streptomycin and Penicillin. Fungicides were used in 0.5% concentration and antibiotic in 25 ugm/ml concentration. In this preliminary screening Captan, Bavistin and Aureofungin were found to give most promising results and thus were selected for further studies. Their activity could be observed from the table XX.

In the next experiment Captan & Bavistin were used in 0.20%, .010%, .005% concentrations while Aureofungin was used in 5 uqm/ml, 10 ugm/ml & 15 ugm/ml concentrations. The results of this experiment are given the in table XXI. From this table it will be

observed that **Rhizopus nigricans** was the most resistant organism and could be inhibited by only Aureofungin and that to in 15 ug/ml concentration. The inhibitory effect of these fungicides on rest of the three fungus were more or less similar. Captan gave the best inhibitory effect followed by Aureofungin & Bavistin successively with refrence to rest of three fungal organisms.

(2) **Test against bacterial organisms**:- **Staphylococcus aureus**, **Streptococcus pyogenes**, **Bacillus sp.**, **Proteus sp.** and **Mycobacterium tuberculosis** were preliminarily screened against eight antibiotic in 25 ugm/ml concentrations. The antibiotic used were Aureofungin, Erythromycin, Kannamycin, Neomycin, Penicillin, Rifamycin, Streptomycin and Tetracyclin. The results obtained are given in table XXII.

Persual of the above table shows that **Proteus sp.** remained uninhibited by any of the above antibiotics. Similarly Aureofungin was ineffective to all the bacterial test organisms. The remaining seven antibiotic gave positive results for their inhibitory effect against the test organisms in various degrees. Penicillin was most active against **S. aureus**, **S. pyogenes** and **Bacillus sp.** While Streptomycin was most active against **M.tuberculosis**.

EXPERIMENT 'B' :- Determination of M.I.C. of fungicides and antibiotics against the selected test organisms.

This experiment was performed in order to get the lowest concentration which could completly inhibit the above microorganisms. The test was performed separately for fungal & bacterial organisms & thus are being discussed below accordingly.

M.I.C of fungicides against fungal test organisms -

During this experiment, Aureofungin, Bavistin and Captan which were most effective during the previous experimental studies, were screened for their M.I.C. against **Aspergillus niger, A. flavus, Penicillium citrinum** & **Rhizopus nigricans** . Bavistin and Captan were used in .005%, 0.010%, & 0.020% concentrations and Aureofungin was used in 1, 5 and 10 ugm/ml concentration. M.I.C. was determined by examining the growth on plates having fungicides in the above dilutions. The lowest concentration in the series of dilution, having no growth after 48hr. was taken as M.I.C. of that fungicides. The results are given in the table XXIII. **Rhizopus nigricans** here again as in previous experiment was not inhibited by either Bavistin or Captan. Aureofungin however inhibited **Rhizopus nigricans** to some extent at 10 ugm/ml concentration.

Therefore M.I.C. for Aureofungin is considered to be between 1 to 15 ugm/ml against **Rizopus nigricans**. This is quite similar to 10 ugm/ml as reported by Thakre & Johri (1973). Against the rest of the fungus M.I.C. was recorded at 10 ugm/ml. Bavistin and Captan however was found to be quite active against rest of the three fungi. For Captan and Bavistin the M.I.C. was found to be 0.010% against **Aspergillus niger**, **A. flavus** and **Penicillium citrinum**. These results are further evident from the plates. Results of these experiments further confirmed the observations made in the previous experiment in which the inhibitory zones were observed. In the previous experiment the greater the concentration, greater was inhibitory zone. The growth recorded in the previous experiment was due to the fact that the inoculum of test organism was made on plain PDA and after 24hr the fungicides were poured in the glass cylinder along the periphery. This was done to find the effect of fungicides on fungal organisms when they were in the active state of growth. In this experiment growth was significantly inhibited because the organisms were incubated on agar medium under influence of fungicides which were mixed while pouring, therefore right from the begining the growth was inhibited.

M.I.C. of antibiotics against bacterial test organisms:-

The experiment was performed as per method discribed by Peleczar et. al., (1977). Increasing dilution series of antibiotic were prepared in peptone broth. A series of each antibiotic constituted fifteen different dilutions.Dilutions used were .05, .075, .1, .15, .2, .3, .4, .15, .2, .3, .4, .5, .6, .9 and 1.0 ugm/ml of the medium. The results obtained against Penicillin, Streptomycin, Tetracyclin, Erythromycin Rifamycin are recorded in table XXIV A and in the plate 4, 5 & 6. In the plate the M.I.C and few other dilutions are shown for obvious reasons. In the above experiment Proteus Sp. was not at all inhibited by any of the above dilutions of the antibiotic tested, Mycobacterium tuberculosis in the above dilution was also not inhibited by Penicillin and Erythromycin. M.I.C. for Staphylococcus aureus, Streptococcus pyogenes & Bacillus sp. against various antibiotic tested were as follows against Erythromycin .6, .05 and .4 ugm/ml, against Pencillin .02,.1 and .02 ugm/ml, against Rafamycin .005,.05 and .070 ugm/ml, atainst Streptomycin .9, .9 and .6 ugm/ml and against Tetracyclin .01, .05 & .1 ugm/ml respectively. Against staphylococcus aureus Rifamycin was most active with an M.I.C. .005 ugm/ml followed by Penicillin .02 ugm/ml Tetracyclin .1 ugm/ml, Erythromycin .6 ugm/ml. Against

Streptococcus Pyogenes Rifamycin, Erythromycin and Tetracycline with M.I.C. of .05 ugm/ml was followed by Penicillin .1 ugm/ml & Streptomycin .9 ugm/ml. Against **Bacillus sp.** Penicillin was most active with M.I.C. of .02 followed by Rifamycin .07, Tetracyclin .1, Erythromycin .4 & Streptomycin .6 ugm/ml. Against **Mycobacterium tuberclosis** Rifamycin was more active with M.I.C. .05 ugm/ml followed by Streptomycin .5 ugm/ml, Erythromycin and Penicillin however could not inhibit **Mycobacterium tuberculosis** in these concentrations. For **Proteus sp.** and **Mycobacterium tuberculosis** raised concentration of those antibiotics were used which could not inhibit these organism in above dilution. In the series of dilutions prepared for this test was in the range of 10, 15, 30, 45, 60, 70, 90, 100 & 200 ugm/ml concentration, The results obtained are given in table XXIV-B. In these results it was found that for **Proteus sp.** M.I.C. of Erythromycin was 90 ugm/ml of Penicillin 200 ugm/ml, Rifamycin 30 ugm/ml, Streptomycin 100 ugm/ml & Tetracyclin 100 ugm/ml. Since these concentrations are very high and could not be used because of their toxicity in blood at these concentrations, therefore these antibiotics are considered to be uneffected and **Proteus sp** is thus regarded as resistant towards them except in Rifamycin where the sensitivety was found to be 30 ugm/ml concentration. M.I.C. obtained in above experiments for

the antibiotics tested with reference to the bacterial organisms are summaried in table - XXV.

These observation of the author are similar to the observation made by Johnb W.Corcorn and Fred (1969). Against Mycobacterium tuberculosis M.I.C. of Penicillin and Erythromycin were found to be 15 and 10 ugm/ml respectively.

EXPERIMENT C :- Effect of selected fungicides and antibiotics on radial growth of test organisms.

Radial growth was observed with Bavistin & Captan in .020%,.010%,.005% concentrations while Aureofungin in 15,10 and 5 ug/ml concentrations. The observations are recorded in table XXVI. Results observed in this experiment confirm the observations of the previous experiment. Since against Rhizopus nigricans, Aureofungin was found to be moderatly inhibitory at 10 ugm/ml concentration as in the previous experiment, thus here it was used at a raised concentration of 15 ugm/ml. also. The results were quite stisfactory and this concentration could be regarded as an M.I.C. Again Captan was found to be most effective followed by Bavistin & Aureofungin for Aspergillus niger, A. flavus & Penicillium citrinum.

Against bacterial test organisms antibiotic used were the same as used in previous experiment 'B'. Data

for <u>Mycobacterium</u> <u>tuberculosis</u> could not be recorded because even in the control it was observed that it is a very slow growing organism and the culture does not become macrosocopically visible until 10 to 17 days or as late as 6 to 8 weeks of incubation. Rest of bacterial organism tested, were same as taken in previous experiment 'B'.

The antibiotic were used in three dilutions only Dilutions selected were in relation to the results and observations made during the experiment for M.I.C. The results are given in table XXVII. These results again confirmed the observations of the previous experiment. All the five antibiotics used significantly inhibited the growth of organism except <u>Proteus</u> <u>sp.</u> which grew almost uneffected. <u>Bacillus</u> <u>sp.</u> was also not much inhibited by Rifamycin in the concentrations used in this experiment. Slight inhibitory effect was however noticed at .05 ugm/ml concentration of Rifamycin and thus the concentration of .070 ugm/ml recomended in the previous experiment appears to be justified.

The general trend observed in this experiment shows that there is a gradual inhibitory effect on growth of the organism tested with a progressive

increase in the concentration of antibiotic. In a few lower concentrations a reverse trend was observed with an increase in incubation period from 0 hr. to 72 hrs.

<u>EXPERIMENT 'D' :- Effect of fungicides on mycelial growth of fungal test organism.</u>

In this experiment only fungal organisms were taken since bacteria do not produce mycelial growth and the dry weight of vegetative cell was negligible, however the effect of antibiotic on their population is considered in next experiment for spore germination.

For Bavistin & Captan concentration used were .030%, .020%, .010% and for Aureofungin was 10, 5 & 1 ugm/ml respectively. The object of this experiment was to see the effect of fungicides in broth culture on the mycelial growth of organism. Since on radial growth mycelium could be observed only superficially but here its actual dry weight can be compared with that of control. The results of this experiment are given in table - XXVIII.

The result obtain are quite interesting in this experiment, especially in case of **Rhizopus nigricans**, Which was found to be uneffected by these fungicides in the previous experiment showed a slight

inhibitory effect by producing mycelial weight, slightly less than that of control both in Bavistin & Captan. While in Aureofungin it produced mycelial weight of 25 mg under the influence of 10 ugm/ml concentration. This is quite significantly reduced from 130 mg dry weight acheived the control. The growth of Aspergillus niger, A. flavus & Penicillium citrinum was completely inhibited by Captan in all dilution tested by Bavistin at .030% concentraion & by Aureofungin at 10 ugm/ml concentration. The inoculum disc might be instrumental in producing a slight growth that was obtained during experiment on Bavistin at M.I.C. In general when compared to the control all the fungicides tested showed inhibitory effect & with the progressive increase in the concentration again the growth gradually decreased.

EXPERIMENT 'E':- Effect of selected fungicides & antibiotic on spore germination of test organisms.

Spore germination of Aspergillus niger, A. flavus, Penicillium citrinum & Rhizopus nigricans were studied on PDA agar disc containting fungicides in desired dilution microscopically at 4, 8 & 12 hrs. intervals. Bavistin & Captan were used in .005%, .010% & .020% concentration while Aureofungin was taken in 5, 10 & 15 ugm/ml concentration. The results are

recorded in table XXIX.

Pnicillium citrinum showed slow spore germination & even in the control experiment germination took place after 24 hrs. Out of these 4 fungal organism usually **Rhizopus nigricans** appeared to be compared to be resistant to the action of fungicides at these concentration. This observation is similar to that of Thakre and Johri (1973 - 1974).

The results obtained in this experiment shows that all organisms exhibit different degree of inhibitory effect under the influence of different concentration. In general it was observed that as the incubation period was increased gradually the rate of germination decreased. It was also observed that the swelling of spores and their germinratrion are two distinct phases of a common but complete phenomenon. The former is less sensitive to the action of fungicides than the latter. This observation was also similar to that of Thakre & Johri (1973 - 74). Captan which appeared to be relatively uneffective on the myelial growth of **Rhizopus nigricans**, gave inhibitorry effect on spore germination. After 12 hrs. interval it reduced the percentage germination to 40%, 50% & 60% in .005%, .010% & .020% concentration respectively. This was quite significantly reduced as compare to that of control & Bavistin, where 100%

spore germination were observed. Aureofungin at he same interval reduced 10% & 66% inhibitation at 10 & 15 ugm/ml concentration respectively with reference to other 3 fungal organisms, results obtained were almost in the similar trend, Captan produced the best inhibitory effect followed by Aureofungin & Bavistin. The activity of Captan & Aureofungin against **Rhizopus** was found to be almost similar to those observed by shridhar (1974). Patel & Rao (1972) found similar results with Captan & Aureofungin against **Alternaria tenius.** Rahanlkar & Neergaard (1969), Thirumalachar (1968), also established the superiorty of these fungicides over, against several other phytopathogenic fungi. Sinha et. al., (1972) have also made an effective control with Aureofungin against **Alternaria citri**. The author observed that Aureofungin reduced the percentage germination of spores and the swelling of spore even at the lowest concentration i.e. lugm/ml. This observation is also similar to that of Cheema & Jeyarajan (1971). The differential action of fungicides on the swelling phase & spore germination as observed by author was also observed by Fletcher (1969); Aderson & Smith (1971) & Gottlieb & Tripathi (1968).

Effect of antibiotic on spore germination of bacterial organisms were studied, following method of Queshel et. al., (1971) & data are recorded in table XXX.

The dilution used were the same as used during the experiment on radial growth. Except **Proteus sp.** rest of the bacteria were found to be sensitive since they were inhibited by concentration below 1.2 ugm/ml. This level is quite suitable as concentration below this level will not produce toxicity in the blood of human beings working in the mill. Against **Proteus sp.** the author observed very high M.I.C. concentrations similar observations were recorded by Korzybski et. al.,(1969) where they observed the M.I.C. of streptomycin to be as high as 420 ugm/ml. Against **Streptococcus pyogenes** no spore germination was found , against concentration, Rifamcycin at 0.05 ugm/ml, Streptomycin at 1.2 ugm/ml & Tetracyclin at .05 ugm/ml concentration, while against Penicillin & Erythromycin only a single colony developed at .05 ugm/ml concentration. Against **Staphylococcus aureus** no colony developed at 0.6 ugm/ml concentration of Erythromycin, 0.025 ugm/ml concentration of Penicillin, 0.005 ugm/ml concentration of Rifamycin, 1.2 ugm/ml concentration of Streptomycin & 0.1 ugm/ml concentration of Tetracyclin.

Against **Bacillus sp.** no spore germination was found at 0.6 ugm/ml concentration of Erythromycin, 0.025 ugm/ml of Penicillin & 0.1 ugm/ml concentration of Tetracyclin while against Streptomycin only single colony developed at .6 ugm/ml concentration. Rifamycin

in the concentration tested could not completely inhibited spore germination of Bacillus sp., though bought an appreciable reduction at .05 ugm/ml concentration. Here on 7 spores germinated as compare to 24 spores of control. Allen (1968) given M.I.C. of Tetracyclin for Bacillus cercus & Streptococcus sp. and also suggested that Erythromycin & Tetracyclin are not effected against Proteus. These observations on Penicillin were noticed against Streptococcus pyogenes by Korzybski et. al. (1969). Atainst Staphylococuus and Streptococcus sp. by Mahendranath et. al., (1976). Streptomycin's activity against Typoid Bacillus, Proteus vulgarius, Staphylococcus aureus & Streptococcus sp. has also been reffered by John W. corcoran & Fred (1969). Erythromycin sensitivity towards Streptococcus, Staphylococcus aureus, Bacillus cereus & Mycobacterium tuberculosis, has been given by Nancy L. oleinick (1969). The authors observations are almost similar to the observation made by these workers.

SECTION IV

ENZYMOLOZICAL STUDIES

CHAPTER - 18

INTRODUCTION

An enzyme (biocatalyst) may be defined as "a protien produced by a living cell which function in catalysing a chemical reaction" Enzymes are characterised by their substrate specificaly. They function in catalysing a single type of transformation of one or few closely related substrates, which implies that a multitude of enzyme are required in metabolism of even a unicellular organism. Enzymes are produced intercellulary or extracellulory. The extracellular enzymes are concerned with reactions outside the organism due to which the decomposition of organic matter is possible (Whitaker 1971).

The range of substances upon which fungi can grow and their potential to decompose a substrate depends upon its enzymatic capabilities. Brown (1915) investigated some active enzymes from fungal mycelia. Several groups of fungi have been reported for the production of various enzymes in connection with organic matter decomposition by Bateman (1969), Chapman et. al., (1975), Coults and smith (1967); Sindu et. al., (1979), Daniel and Dube (1983); Muthal and Saksena (1973); Goel & Mehrotra (1973, 1975); cappelline (1965); Bhatia and Chohan (1970); Norkans and Fuller (1942).

Ascomycetes & fungi inperfecti play an important role as plant decomposers in soil & several fungi of these by groups are involved in deterioration of cellulolytic materials. Aspergilli, amongst decomposers is a very prominent group in decomposing various kind of organic matters such as leaf litter, clothing, paper, wood etc. Virtually all fungi can utilize glucose but increasing complexity of the substrates requires production of particular enzymes that leads to the loss in coherence in the cells & thus make the substrate palatable to soil funna and other micro-organisms. Some spices are specially equipped in this respect than others.

Cellulose form the most prominent part of plant remains, particularly the man-made litter such as textile and paper products. Cellulose is chemically polymer of B-1, 4 linked glucose residues. It is localised in the cell wall, both in primary & secondary depositions where it is found in the form of simple chains & then in to larger structures the microfibrils. The microfibrils remains embedded in an amorphous matrix of gel, consisting of protiens, natural sugars, the hemicellulose togather with other uronically rich polysaccharides, pectin etc. In this form the cellulose is insoluble. Cellulose and the related substances undergo hydrolysis on enzyme action. The group of

enzymes involved are the "celluloses". Cellulose are thus very important in nature for the degradation of substances particularly dominated with cellulose. More than one component of cellulose system is involved in the degradation of cellulose (Reese et. al.,(1957); Li, et. al., (1965); Mendels and Reese (1965); Selby and Maitland, (1967); Lusis & Backer (1973); Eriksen and Guksayar (1976); Sternberg et. al., (1977), Whitaker (1971); Batman (1964).

Large number of fungi have been reported to be cellulolytic Reese (1949); Gascoigne & Gascoigne (1960); Garrett(1962); Yamane et. al., (1965); Mendels & waber (1969); Oso (1978); Erikson & Rzedowsski (1969); Agarwal (1969); C.L.Fergus (1964); Bellamy (1974); Crawford & crawford (1976). These fungi produce cellulose when grown on substrates having cellulose. Blum (1952) studied the enzymatic degradation of cellulose fibres Norkans (1950.b) investigated that **Trichoderma fumosum** in contact with cellulose produces this enzyme extra cellularly in culture on comparing the fungal population of soil with soil amended with cellulose source. Jensen (1931) found enchanced related fungi in latter, Van Iterson (1904) used soaked filter paper in mineral salts, Tribe (1957) buried strips of cellophane paper struck to cover slips, Webley & Duff (1962) buried pellets of Kaolinite incorporating the

cellulose & Pugh (1966) selected whatman's chromatographic cellulose paper in soil amendment to observe the cellulolytic fungi. The biodegradation of cellulose has also been reviewed by some other workers, Reese *et. al.*, (1950, 1963): Siu (1951); Hogg (1966); Selby (1961); Norkans (1963); Rosenberg (1978); Norkans & Ramby (1956); Fergus (1969); Dwivedi & Singh (1974); Walsh & Stewart (1971); Wood and Macrae (1972); Rai (1969). In all cases cellulosic decay results due to the activities of enzymes celluloses secreted by micro-organism.

Physical and cultural conditions influence the cellulose production (Norkans 1963). Youatt (1958) reported that high rate of shaking (120 C/min) had a deletirious effect on the cellulose concentrations obtained from *sachybotrys atra.* Cellulose production by root infecting ascomycetes *Rhizina inflata* has been obtained only in stationary cultures and not in moderately agitated ones, nor in those which are gently bubble - aerated, even in small vessels (Norkans & Hammarstrom 1963).

The classical cellulose enzyme system of Pringsheim (1912) is made up of at least two entitics designated as C_1 & C_x respectively. The same were also refferred by other workers (Siu & Reese 1953; Bateman 1964). The celluloses which can hydrolyse or alter the

native cellulose have been considered to produce an enzyme reffered to as C_1 enzyme. There are several views as far as the action and nature of celluloses is concerned. Previously it was believed that a simple enzyme degrads insoluble cellulose to glucose. Kooiman (1957) and Simson et. al..(1958) reported that one enzyme degrades insoluble cellulose to cellobiose and other produce glucose from it. The current concept summarised by Oso (1978) revealed that degradation of native cellulose involves two types of enzyme complex C_1 & C_x, C_1 acts on crystalline cellulose in such a way that subsequent action by the Cx enzyme may become possible. The Cx complex of enzyme hydrolysing the β-1-4 glucosidic bonded in the cellulose molecule does not hydrolyse crystalline cellulose in the absence of the C_1 enzyme.

Besides cellulose other polysaccharides specially pectic substances are also found in plant remains and their degradation has a prominent role in the biodegradation.

The biodegradation of pectic enzyme has been reviewed by author like wood (1960), (1955); wang et. al. (1971); Batemen and Millar (1966); Demian and Phaff (1957). Pectic substances are complex polysaccharides composed of galacturonic acid units, bound to one another in long chain. The carboxyl of the galacturonic

acid building block may be partially or completely esterified with methyl groups may be partially or entirely neutralized by various cations. Pectic material contribute the principal intercellular cement or the middle lamellar structure in the plant tissue. This substances is incorporated with in the matrix containing microfibrils of cellulose and other polymers.

The enzymes are produced by many saprophytic micro-organism, which disintegrate plant tissue and by many plant pathogenic microbes which use pectic enzyme as a " spreading factor." Large number of fungi have been reported to produce pectic enzymes, Ayer et. al.,(1966); Kadirvel et. al.,(1967); Honcock et. al., (1964); Bateman (1966); Kannaiyan et. al., (1975). The two most easily recognized chemical changes brought about in pectic substances by enzymes are the hydrolysis of methyl ester and the glycosidic bonds. These changes are catalysed by pectine-esterase (pectase) and polygalacturonase respectively. The occurence of wide range of pectic substances in nature involve a complex of pectolytic enzyme-pectic methyl esterase (PME), Pectin methyl galacturancese (PMG), Polygalacturonase (PG) and transeliminase or lyases, depending upon the nature of the substrate under going enzyme action.

Since the enzyme cellulolytic & pectolytic both play such as important role in tissue degradation, their study is of utmost importance while studing the microbial dynamisms of cotton fibres.

But main attention has been given by the auther towords cellulolytic enzymes. As the fibres contain about 94% cellulose. The organism which form the part of dominant fungal population during the earlier studies were selected and the enzymatic analysis was done in broth culture having cotton as a sole carbon source. The analysis was done at three different stages of fungal growth in vitro. In these series of experiments it has been the to determine whether or not the test fungal species can bring successful degradation of cotton fiber and to find out the organism which has the best enzyme system for the degradation process.

Goel & Mehrotra (1973-74); Grover (1963), (1964); Ramaraj & Vidyasekaran (1986),Kannaiyan (1988) Gaur et. al., (1988); Mehta (1979); Mehta and Mehta (1988); have studied the effect of fungicides/antibiotic on enzyme activity of various fungi. They have reported the fungicides inhibit the production and activity of enzyme. But most of these work were conducted on the organisms causing plant diseases and the object of these studies were to control plant diseaases. The

author conducted these studies not in relation to the diseases of cotton but to explore the enzymatic capabilities of the organisms which were so intimately associated with cotton fibres Bacterial organism selected during isolation studies & used in the previous section were not selected for the studies in this section because most of them were not true saprophytic organisms of cotton. Based on the above back ground of the effect of fungicides in their M.I.C. on enzyme activity was also studied.

CHAPTER - 19

EXPERIMENTAL

To explore the enzymatic capabilities of the selected fungal organisms still cultures were raised on broth mediums. Both cellulolytic and pectolytic enzymes were studied using standard Fenske- Ostwald Viscometric procedure. Fungal organisms were grown on broth cultures media having cotton as the sole carbon source and the enzyme assay was done from culture filtrate obtained after desired period of growth in 100 ml flasks containing 35ml of the sterilized medium. Inoculations were done in each case with a 7mm agar disc of the test organisms cut from the margin of freshly grown colony. The flasks were incubated at 32 degree C for a period of 6, 12 and 18 days. Triplicates were taken for each culture, pH was adjusted to 4.5 and controls were run side by side. Culture filtrates were obtained by filtering under suction in Buchner`s funnel. The cell free culture filtrate were than cetrifuged at 4000 RPM for 20 minutes. The surpernatant liquid was than taken as enzyme extract or frozen for the purpose of storage, if required for latter use. The enzyme preperation obtained was used for testing the presence & activity of cellulase (Cx) & pectolytic (PMG & PMTE) enzymes. The enzyme assay was done viscometrically in Fenske Ostwald viscometers (Cepellini 1966, Agarwal 1971). The enzyme reaction

mixture consisted of :-

Substrate	-	3.5 ml
Distilled water	-	1.5 ml
Mcllvaine's Buffer (pH5.5)	-	1.5 ml
Enzyme preparation.	-	1.5 ml

For determining cellulase substrate used was a 1.2% solution of carboxy methyl cellulose (CMC). For PMG and PMTE the substrate was 1.2 % solution of citrus pectin. For PMTE the buffer of pH 8 was used.

Experimental procedure followed was as follows :-

1) Ostwald viscometers were fixed vertically in a water bath where the temperature was adjusted to 30 degree C.

2) The enzyme reaction mixture was than poured in the stock bulb in the same sequence as given above.

3) The efflux time for 8 C.C. of this enzyme reaction mixture was determined at an interval of 5, 15, 30, 60 and 90 minutes (This is taken as reaction time). After pouring the reaction mixture in viscometer.

4) Enzyme reaction mixture with autoclaved enzyme extract was run as control. Efflux time for 8 C.C. of water was also noted of each viscometer.

The percentage loss in viscocity were calculated according to following formula :-

$$\text{Percentage loss in viscosity} = \frac{ETo - ETt}{ETo - ETw} \times 100$$

Where,

ETo = Efflux time at O'hr.

ETt = Efflux time of reaction mixture at time t'.

ETw = Efflux time of water.

The relative enzyme activity REA was calculated as follows :-

$$REA = \frac{1000}{t \text{ at } V_{50}}$$

Where 't'at V_{50} represents the time in minutes to bring about the 50% loss in viscosity of the initial. Values of percentage loss in viscosity were plotted against reaction time, from these curves time taken to bring about 50% loss in viscosity was noted. Where 50% loss in viscosity was not obtained after 90 minutes reaction time, the value obtained at 90 minutes of reaction time was used to evaluate the time taken to obtain 50% loss in viscosity.

The studies were conducted in two parts. In the first cellulolytic enzymes and in the second pectolytic enzymes were studied.

PART I : <u>Studies on cellulolytic enzymes</u> :-

Cellulolytic enzyme production were conducted

in the following manners :-

(1) Effect of media & age of culture on production of cellulolytic enzymes.

(2) Effect of fungicides on the production of cellulolytic enzymes of test fungus.

(1) Effect of media and age of culture on production of cellulolytic enzymes.

Isolation of culture filtrates to be used as enzyme preparation was obtained according to procedure described above. Two different media i.e. Basal media (Hogland *et. al.*, 1959) and Glucose asparagine media (Cole 1956) were used having the following composition.

Basal medium (Hogland *et. al.* 1959)

D. glucose	-	5.0 gm.
Asparagine	-	0.7 gm.
KH_2Po_4	-	2.0 gm.
$MgCl_2\ 6H_2O$	-	0.05 gm.
$Mncl_2\ 6H_2O$	-	0.005 gm.
$Fe\ cl_2$	-	0.005 gm.
$Zn\ cl_2$	-	0.005 gm.
L. Methionine	-	0.095gm.
Distilled water.	-	1 litre
pH	-	5.5

Glucose asparagine medium (Cole 1956).

Glucose	-	10.0 gm.
Asparagine	-	5.0 gm.
KH_2PO_4	-	1.0 gm.
$MgSO_4 7H_2O$	-	0.5 gm.
P^H	-	5.5
Distilled water	-	1 litre

In both the medium glucose was replaced with cotton fibre as a sole carbon source.

Procedure for extraction of enzyme preparation and assay of enzyme was the same as described above.

(2) <u>Effect of fungicides or production of cellulolytic enzyme activity of test fungus</u>:-

Glucose asparagine medium was used in this study as high cellulose activity was recorded in the culture filtrate of this medium in the above experiment. Bavistin, Captan and Aureofungin were used in this experiment as these were selected from the studies of Sec. III. Bavistin and Captan in 0.010% concentration and Aureofungin in 10 ugm/ml concentration were added to the medium after autoclaving. Procedure for raising culture filtrate, extraction of enzyme and assay of enzyme was the same

as described above.

PART II: Studies on pectolytic enzymes :-

Pectolytic enzymes studieds were pectin methyl galacturonase (PMG) and Pectin methyl transeliminase (PMTE). For both the experiments, age of culture and effect of fungicides, culture filtrates were raised on glucose asparagin medium. Analysis for the above pectolytic enzyme under the influence of age of culture and fungicides were done according to procedure described above. The same fungicides were used in the same above concentrations.

CHAPTER - 20

RESULTS AND CONCLUSIONS

The fungi selected for enzymatic studies were *Aspergillus niger*, *A. flavus*, *Penicillium citrinum* and *Rhizopus nigricans.* The cellulolytic and Pectolytic enzyme production of these organisms were determined in terms of percentage loss in viscosity and REA units at three different incubation periods i.e. 6, 12 and 18 days. The results obtained in the various experiments are given below :-

PART I : STUDIES ON CELLULOLYTIC ENZYMES:-

EXPERIMENT 'I' - Effect of media and age of culture on cellulase enzyme activity.

The effect of culture media and age on the production of cellulase by *A. niger*, *A. flavus*, *P. citrinum* and *R. nigricans* were analysed and data were recorded for percentage loss in viscosity and REA.

It is clear from the data recorded that glucose asparagine medium favoured the production of cellulase more as compared to basal medium for all the fungal organisms tested. The REA value was found to be maximum in case *A. niger* followed by *A. flavus*, *R. nigricans* and *P. citrinum* after 6 days of incubation. As the incubation period was increased to 12 and 18 days the results varied. *A. niger*, *A. flavus* and *R.*

TABLE - XXXI

Cellulase activity of selected fungi in glucose aspragin medium at different incubation period

(i) Percentage loss in viscosity of reaction mixture at time `t'
(ii) Relative enzyme unit

S.NO.	Organisms	6 Days						12 Days						18 Days					
		% Loss in viscosity					REA	% Loss in viscosity					REA	% Loss in viscosity					REA
		5	15	30	60	90		5	15	30	60	90		5	15	30	60	90	
1.	Aspergillus niger	29.6	48.3	57.8	63	69.7	55.5	25	47.6	58.2	68.9	72	52	23	45	60	70	70.8	50
2.	Aspergillus flavus	15	26.9	35	48.7	55.9	14.2	16.4	28.4	37	47.6	52.3	12.8	19.8	30	39	45.7	50.3	11.1
3.	Penicillum citrinum	5	10.8	17	25.5	30.6	6.8	15.8	30.9	35.6	49	56	16.12	20.8	32.2	40	50	61.3	16.6
4.	Rhizopus nigricans	18	27.6	33	45	56.4	13.6	11	23	32.8	43.5	50	11.1	10	14	35.6	42	49	10

TABLE - XXXII

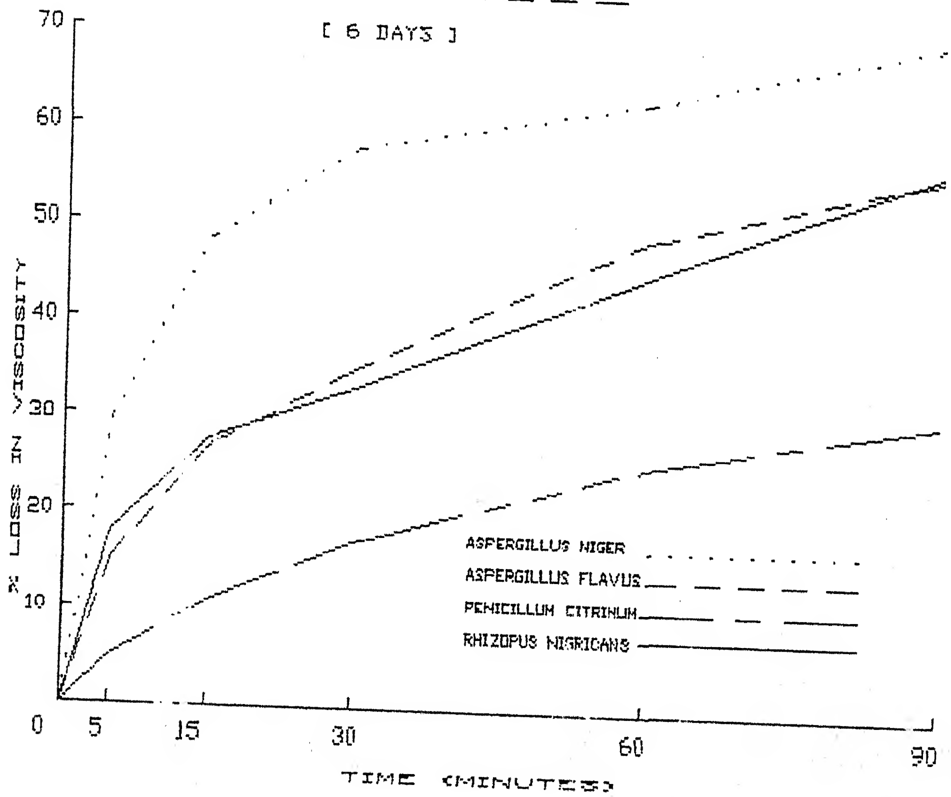
Cellulase activity of selected fungi in basal medium

(i) Percentage loss in viscosity at time `t'
(ii) Relative enzyme unit

S.No.	Organisms	6 Days						12 Days						18 Days					
		% Loss in viscosity					REA	% Loss in viscosity					REA	% Loss in viscosity					REA
		5	15	30	60	90		5	15	30	60	90		5	15	30	60	90	
1.	Aspergillus niger	22.77	35.4	35.9	36.8	58.2	15.3	15.8	30.5	40.4	46.8	50.25	11.1	8	24.8	30.6	36.2	39.4	8.7
2.	Aspergillus flavus	14.8	15.7	14.7	30.6	38.3	8.5	6.87	15.78	18.66	26.54	30.55	6.7	5.1	12.1	15.6	20.4	25.2	5.6
3.	Penicillum citrinum	4.49	9.89	15.61	25.99	27.11	6.02	12	16	19	21	28	6.25	8	17	21	23	31	6.8
4.	Rhizopus nigricans	15	26	30	36	42	9.3	11	21	28	33	37	8.2	8	11	19	26	27	6

CELLULASE ACTIVITY OF SELECTED FUNGI IN GLUCOSE ASPARAGIN MEDIUM

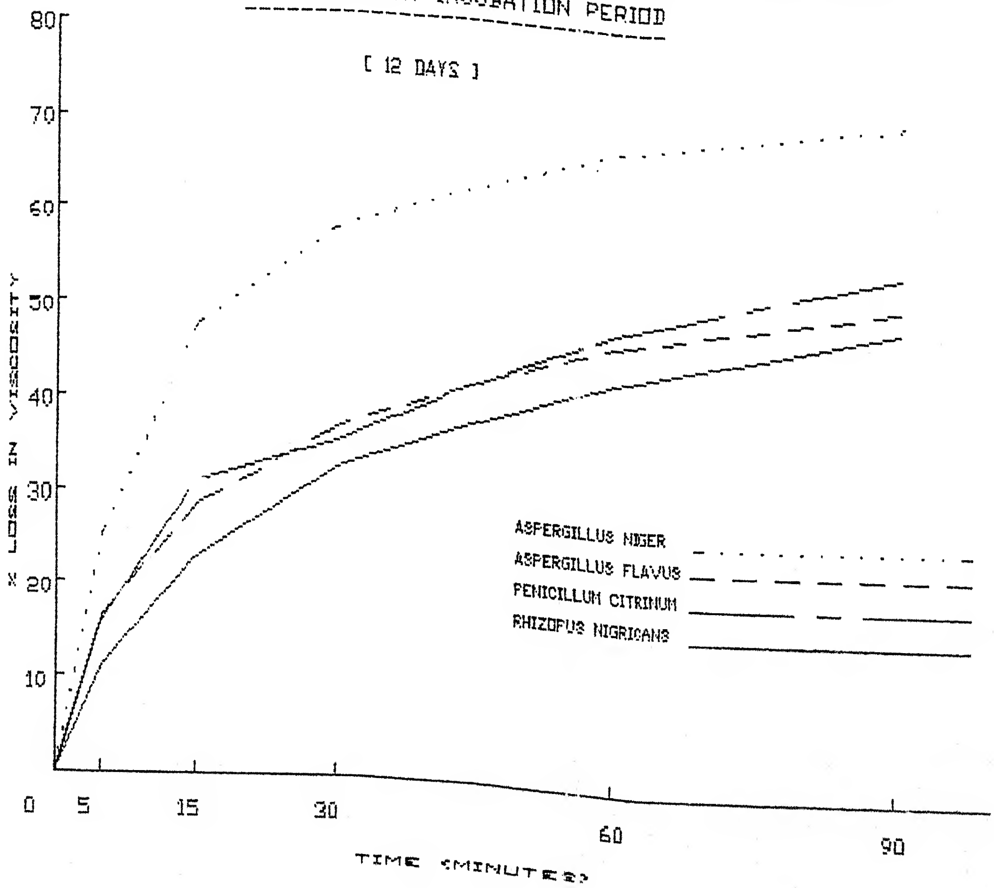
AT DIFFERENT INCUBATION PERIOD



(FIGURE 17 'A')

CELLULASE ACTIVITY OF SELECTED FUNGI IN GLUCOSE ASPARAGIN MEDIUM

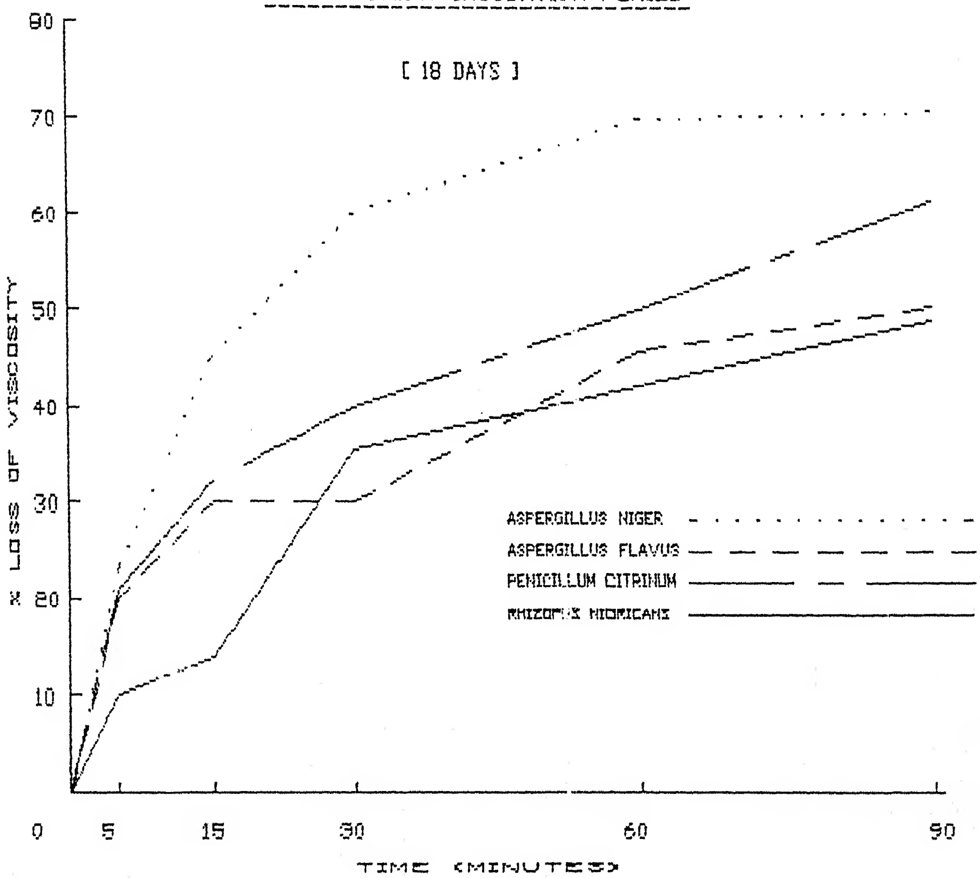
AT DIFFERENT INCUBATION PERIOD



(FIGURE 17 'B')

CELLULASE ACTIVITY OF SELECTED FUNGI IN GLUCOSE ASPARAGIN MEDIUM

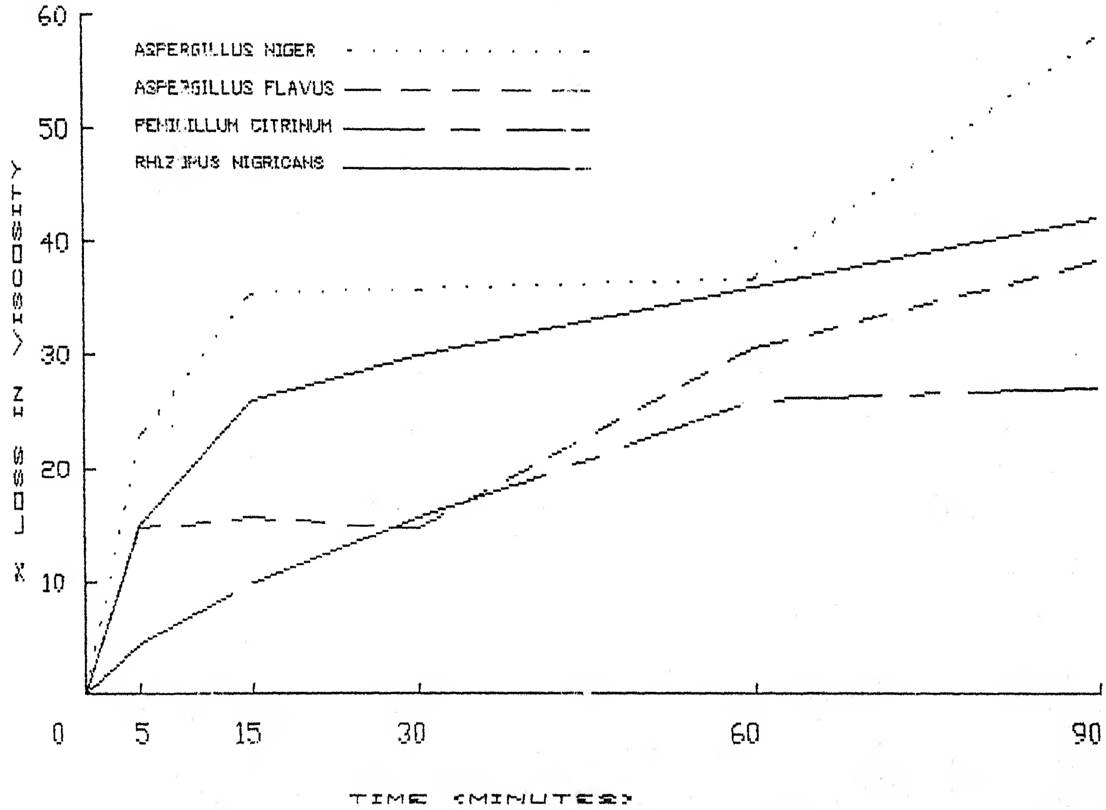
AT DIFFERENT INCUBATION PERIOD



(FIGURE 17 'C')

CELLULASE ACTIVITY OF SELECTED FUNGI IN BASAL MEDIUM

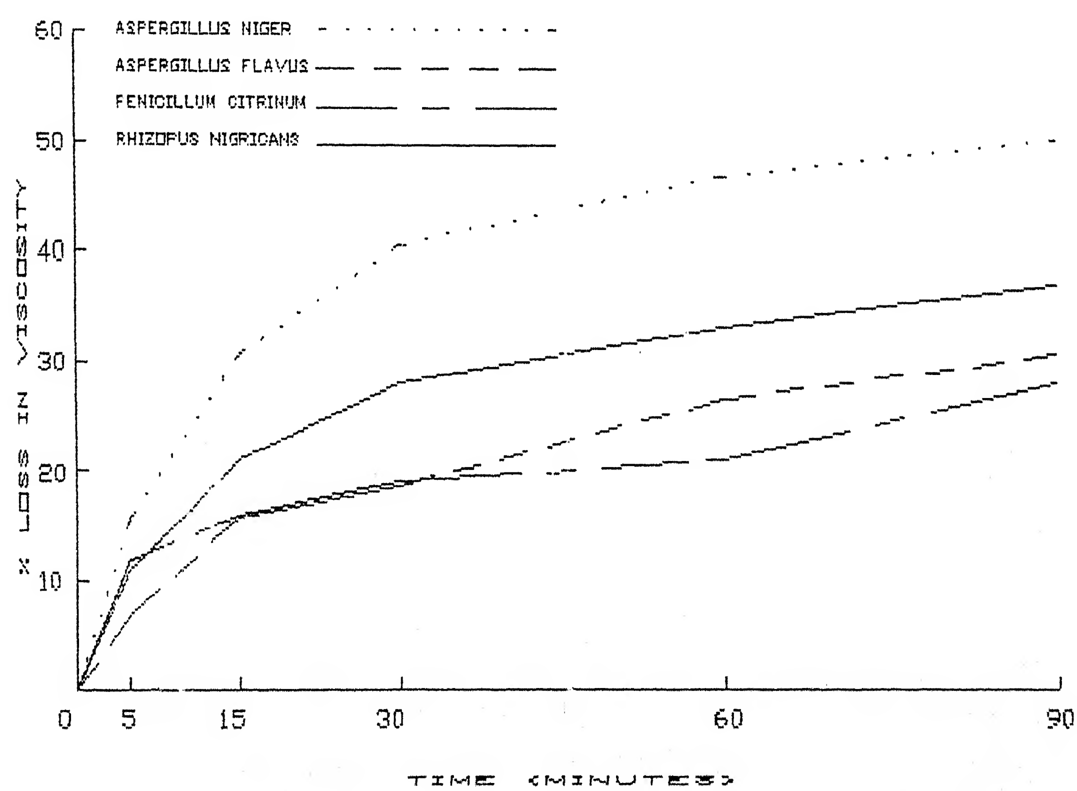
[6 DAYS]



(FIGURE 18 (A))

CELLULASE ACTIVITY OF SELECTED FUNGI IN BASAL MEDIUM

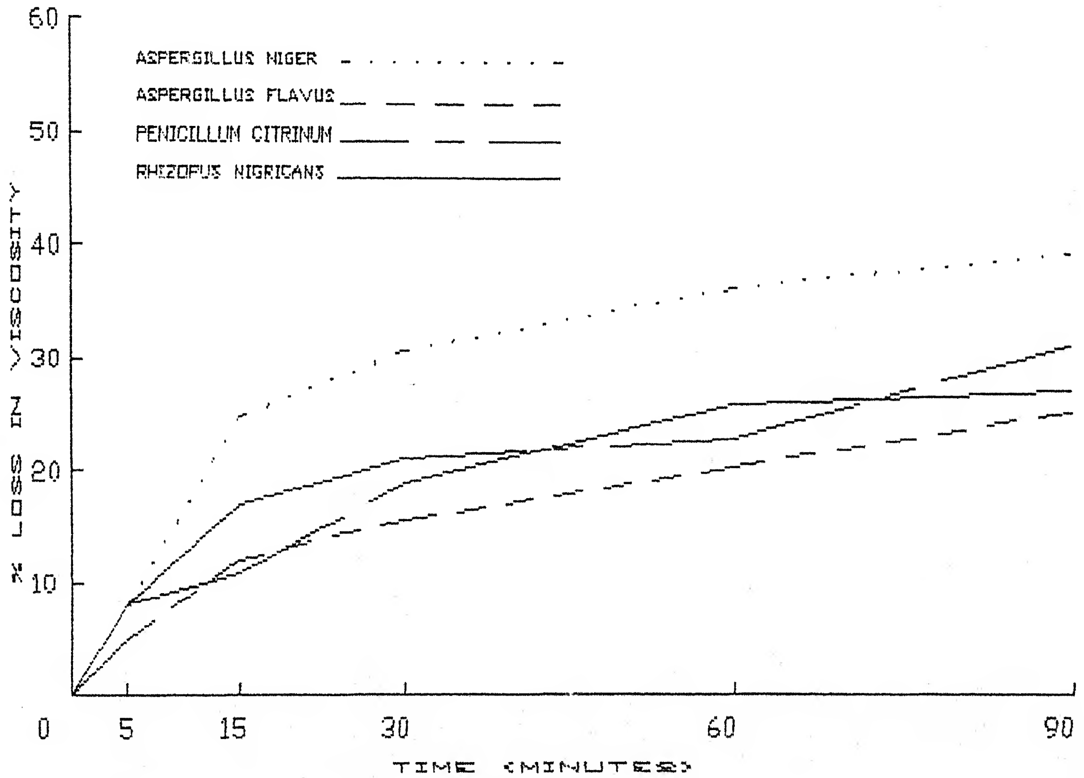
[12 DAYS]



(FIGURE- 18 'B')

CELLULASE ACTIVITY OF SELECTED FUNGI IN BASAL MEDIUM

[18 DAYS]



(FIGURE-18 'C')

nigricans reduced their enzymatic activity with rise in the incubation periods. Thus for these organisms, the best enzymatic activity was obtained after 6 days of incubation, for **P. citrinum** the enzymatic activity increased with increase in incubation period and maximum activity was obtained after 18 days of incubation. The enhancement in the enzymatic activity was more pronounced in glucose asperagine medium when the incubation was raised from 6 to 12 days. When this activity is compared to the activity of 18 days incubation period it is evident that the activity is only slightly raised. These results are clear in the datas given in table XXXI and XXXII and Fig.17 A. B. C. and 18 A. B. C.

EXPERIMENT 'II' - EFFECT OF FUNGISIDES ON PRODUCTION OF CELLULASE ENZYME BY SELECTED TEST ORGANISMS.

The datas given in the table XXXIII clearly shows that out of the four fungal test organisms against Bavistin, Captan and Aureofungin, only **Rhizopus nigricans** could develop considerable enzymatic activity. Out of the three fungicides tested Captan and Aureofungin caused a complete inhibition of mycelial grawth therefore also of cellulose production in **A. niger**, **A. flavus** and **P. citrinum**. Bavistin on the other hand was comparatively least toxic because some

TABLE - XXXIII

Effect of fungicides on cellulase production of fungal test organism

Organism	Fungici- -des used	Conc. of fungicide	6 Days						12 Days						18 Days					
			% Loss in viscosity					REA	% Loss in viscosity					REA	% Loss in viscosity					REA
			5	15	30	60	90		5	15	30	60	90		5	15	30	60	90	
Aspergillus niger	Bavistin	.010%	0	0	2	3	5	1.1	0	0	3	5	7	1.5	0	0	1	2	4	0.8
	Captan	.010%	-	-	-	-	-				-	-	-	-			-	-	-	-
	Aureo- -fungin	10 ugm/ml	-	-	-	-	-				-	-	-	-			-	-	-	-
Aspergillus flavus	Bavistin	.010%	0	0	1	3	6	1.3	0	2	5	8	11	2.4	0	2	4	6	8	1.7
	Captan	.010%	-	-	-	-	-				-	-	-	-			-	-	-	-
	Aureo- -fungin	10 ugm/ml	-	-	-	-	-				-	-	-	-			-	-	-	-
Penicillum citrinum	Bavistin	.010%	0	0	2	4	6	1.3	0	0	2	4	7	1.5	0	0	1	4	6	1.3
	Captan	.010%	-	-	-	-	-				-	-	-	-			-	-	-	-
	Aureo- -fungin	10 ugm/ml	-	-	-	-	-				-	-	-	-			-	-	-	-
Rhizopus nigricans	Bavistin	.010%	16	23	25	35	47	10.	10.	17	21.5	28	37.7	8.2	9.9	11.	19	23.3	32.2	7.1
	Captan	.010%	11	19	21	27	36	8	9	20	23.6	25	32	7.1	10	14	19	24	30	6.6
	Aureo- -fungin	10 ugm/ml	0	1	4	7	10	2.2	5	10	13	16	20	4.4	5	9	11	13	15	3.3

\- ---> Complete inhhon

mycelial growth of these three test organism were obtained and this resulted in developing some enzymatic activity. However this activity was quite insignificant as compared to the activity obtained on the medium without fungicides as shown in the table XXXI and XXXII. On <u>Rhizopus nigricans</u> maximum reduction of cellulase production was caused by Aureofungin followed by Captan. Bavistin however did not supressed the cellulase production of this fungus in the concentration used. The fungi toxic effect of Aureofungin was also found on the vegetative growth of <u>R. nigricans.</u> In general all the three fungicides tested showed considerable reduction in cellulose activity on all the organisms tested except for Bavistin in <u>R. nigricans</u>. The inhibitory effect of the fungicides were more pronounced in cultures of 6 days incubation period. As the incubation period increased their enzymatic activity increased.

<u>PART 2 STUDIES ON PECTOLYTIC ENZYMES</u>:-

<u>EXPERIMENT 'I'- EFFECT OF AGE OF CULTURE ON PECTOLYTIC ENZYME ACTIVITY</u>.

Data recorded in table XXXIV and XXXV and Fig. 19 A.B.C. & 20 A.B.C. shows that the test fungus produced PMG and PMTE on broth culture in glucose asparagin medium with cotton as a sole carbon source.

TABLE - XXXIV

PMG (Pectin methyl galacturonase) activity of test organism at different incubation period

S.No.	Organisms	6 Days						12 Days						18 Days					
		% Loss in viscosity					REA	% Loss in viscosity					REA	% Loss in viscosity					REA
		5	15	30	60	90		5	15	30	60	90		5	15	30	60	90	
1.	Aspergillus niger	20	25	31.2	37.5	39	6.25	15.3	23	30.15	35	39	8.6	14.1	24.7	25	28.5	31.5	7.04
2.	Aspergillus flavus	11.22	13.3	17.7	22.2	31.1	6.89	13	19	25	29	35	7.8	13	18	21	24.7	31.8	7.09
3.	Penicillum citrinum	9.54	15	19	23.6	28.18	6.25	5	8	14	18	25	5.5	4	8	10	16	28	6.25
4.	Rhizopus nigricans	11.76	17.6	35.2	47	52.4	11.6	12.5	18	31	45.2	48.7	10.7	10	17	27.9	38	41	9.1

TABLE - XXXV

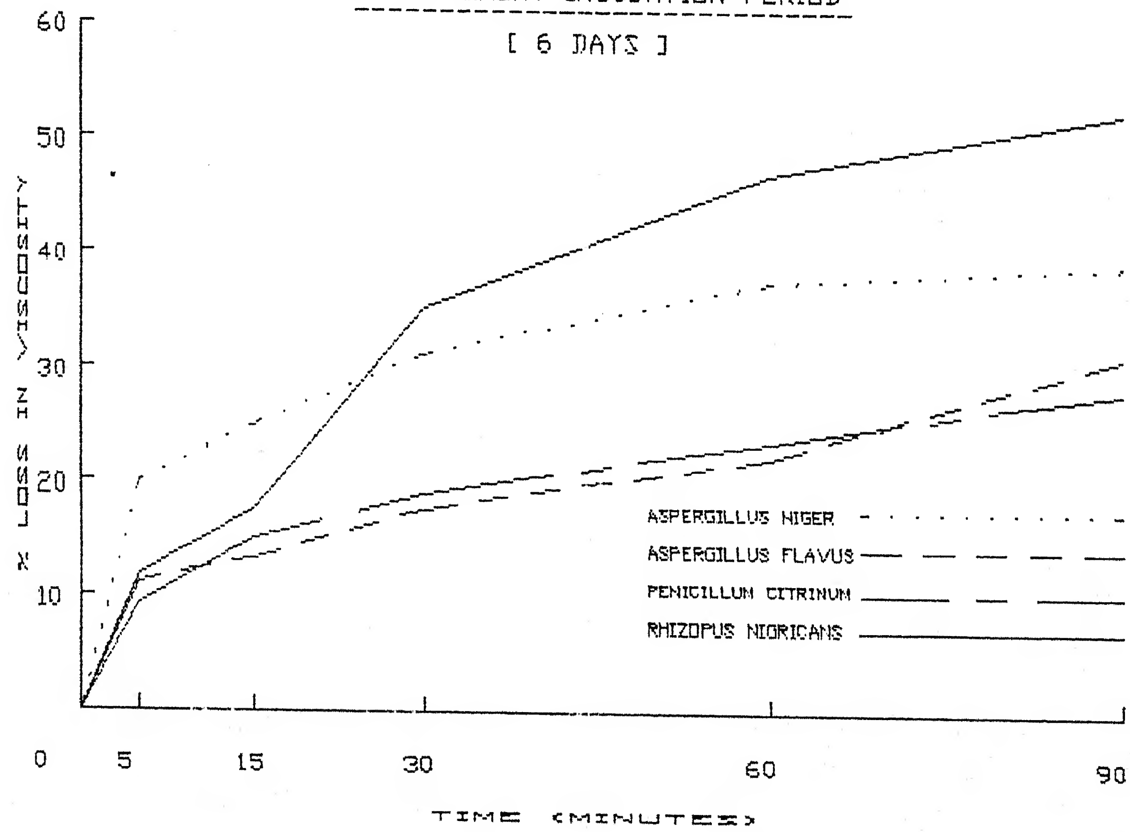
PMTE (Pectin methyl transeliminase) activity at different incubation period in glucose asparagin medium

S.No.	Organisms	6 Days						12 Days						18 Days					
		% Loss in viscosity					REA	% Loss in viscosity					REA	% Loss in viscosity					REA
		5	15	30	60	90		5	15	30	60	90		5	15	30	60	90	
1.	Aspergillus niger	0	8	11	21	34	7.5	31	48	43	49	52.5	11.6	15.2	20.7	25	35.6	40.6	8.9
2.	Aspergillus flavus	7.5	10	12.5	17.5	22.5	4.9	25	28	32	37	49	10.9	18	21	27	31	37	8.2
3.	Penicillum citrinum	11	18	23	30	31.4	6.8	17	21	28	33	38.4	7.5	15	19	25	31	34	7.4
4.	Rhizopus nigricans	18.5	28.5	33.3	36	38	8.4	10.8	20.8	36.9	41.3	45.6	10	9.8	11.8	34	38.2	42	9.3

PMG (PECTION METHYL GALACTURONASE) ACTIVITY OF TEST ORGANISM

AT DIFFERENT INCUBATION PERIOD

[6 DAYS]

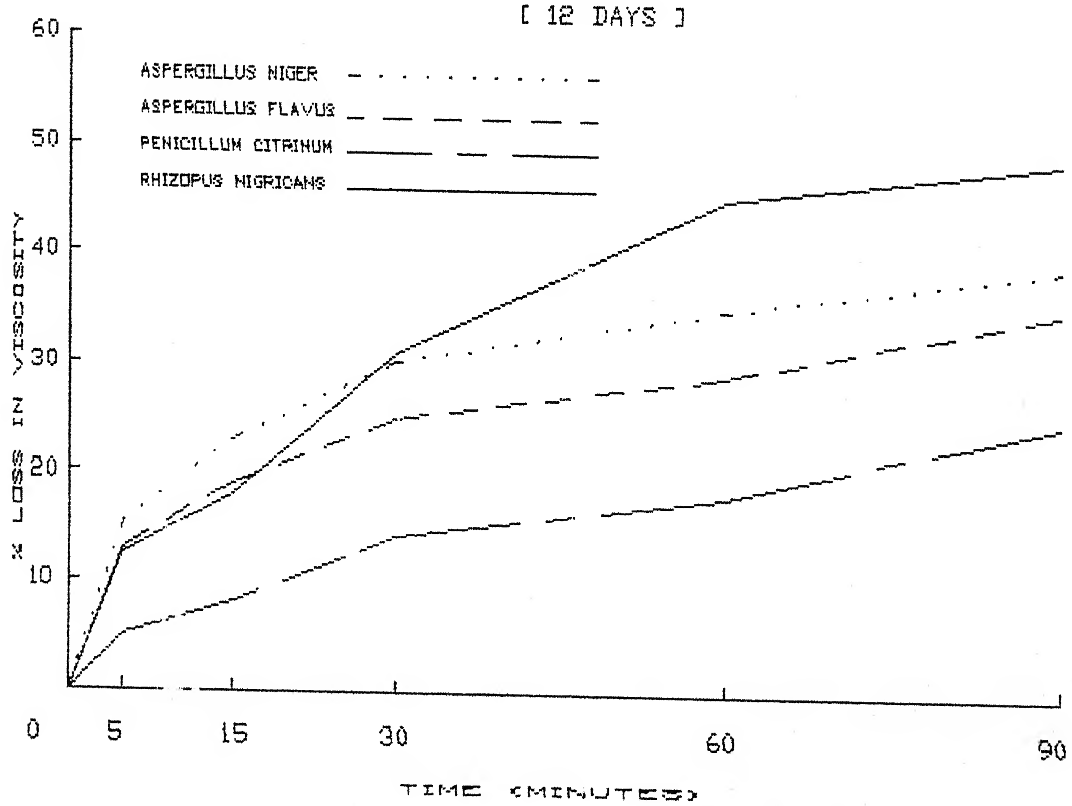


(FIGURE-19 'A')

PMG (PECTION METHYL GALACTURONASE) ACTIVITY OF TEST ORGANISM

AT DIFFERENT INCUBATIONPERIOD

[12 DAYS]

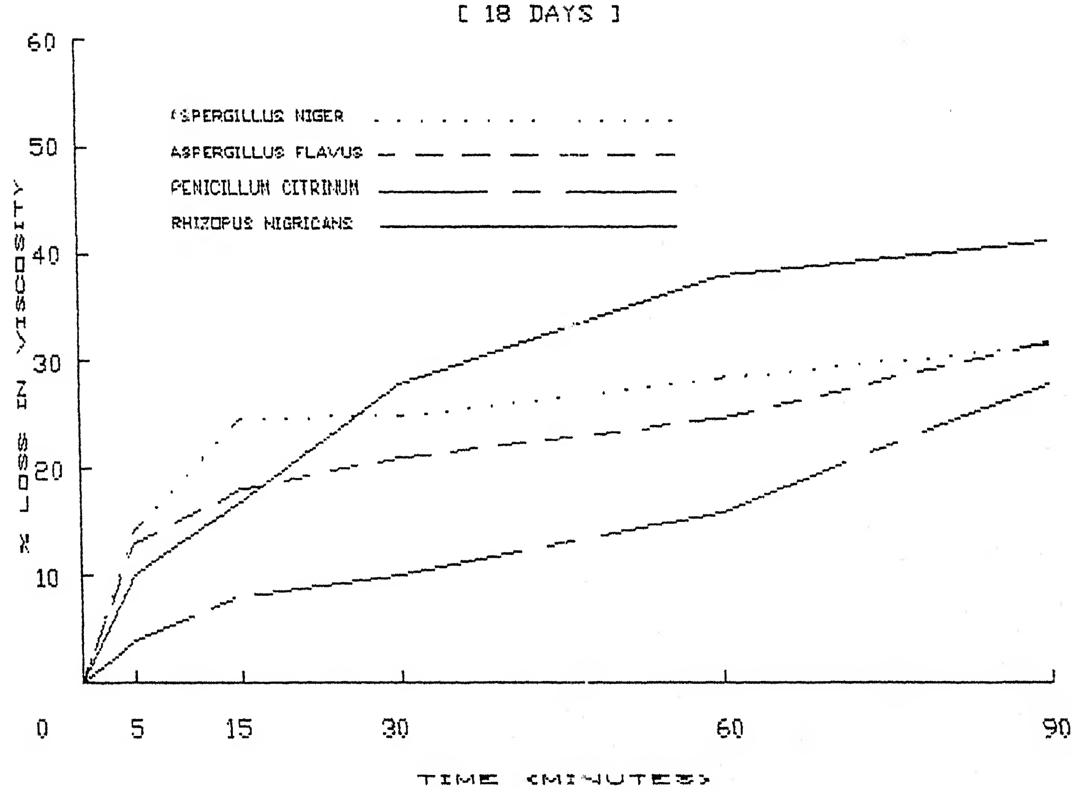


(FIGURE-19 'B')

PMG (PECTION METHYL GALACTURONASE) ACTIVITY OF TEST ORGANISM

AT DIFFERENT INCUBATION PERIOD

[18 DAYS]

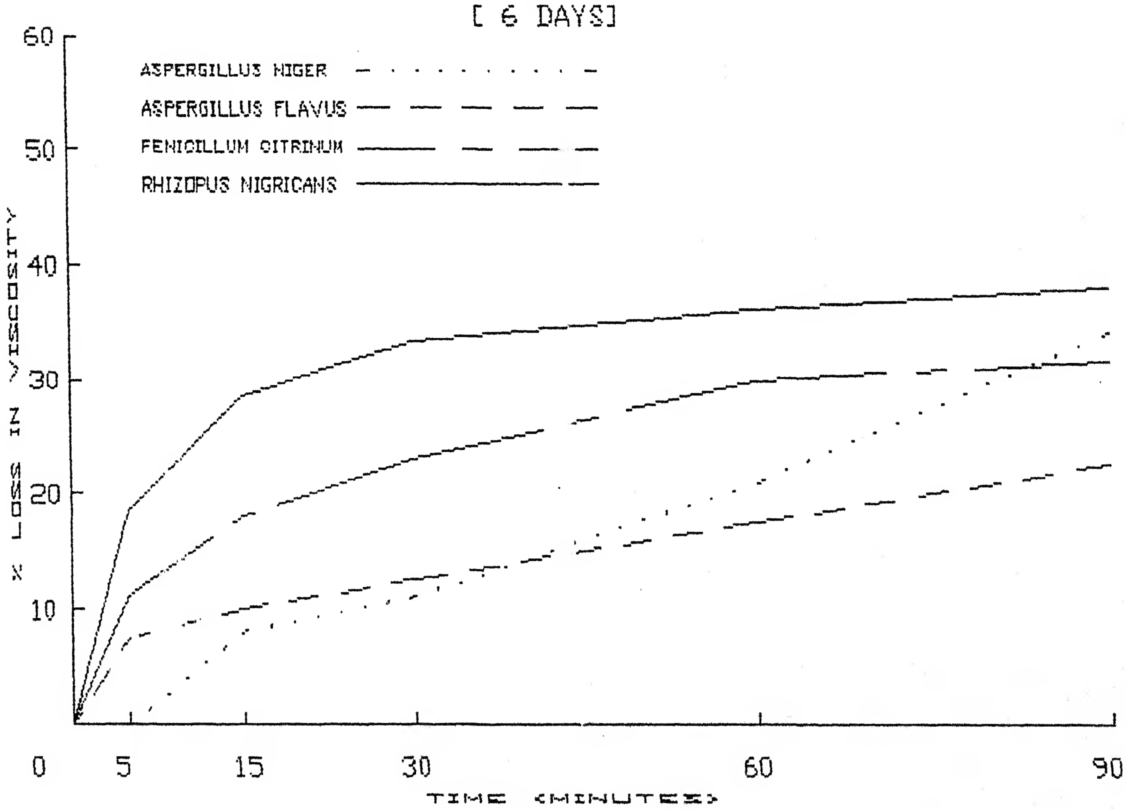


(FIGURE-19 'C')

PMTE (PECTION METHYL TRANSELIMINASE) ACTIVITY AT DIFFERENT

INCUBATION PERIOD IN GLUCOSE ASPARAGIN MEDIUM

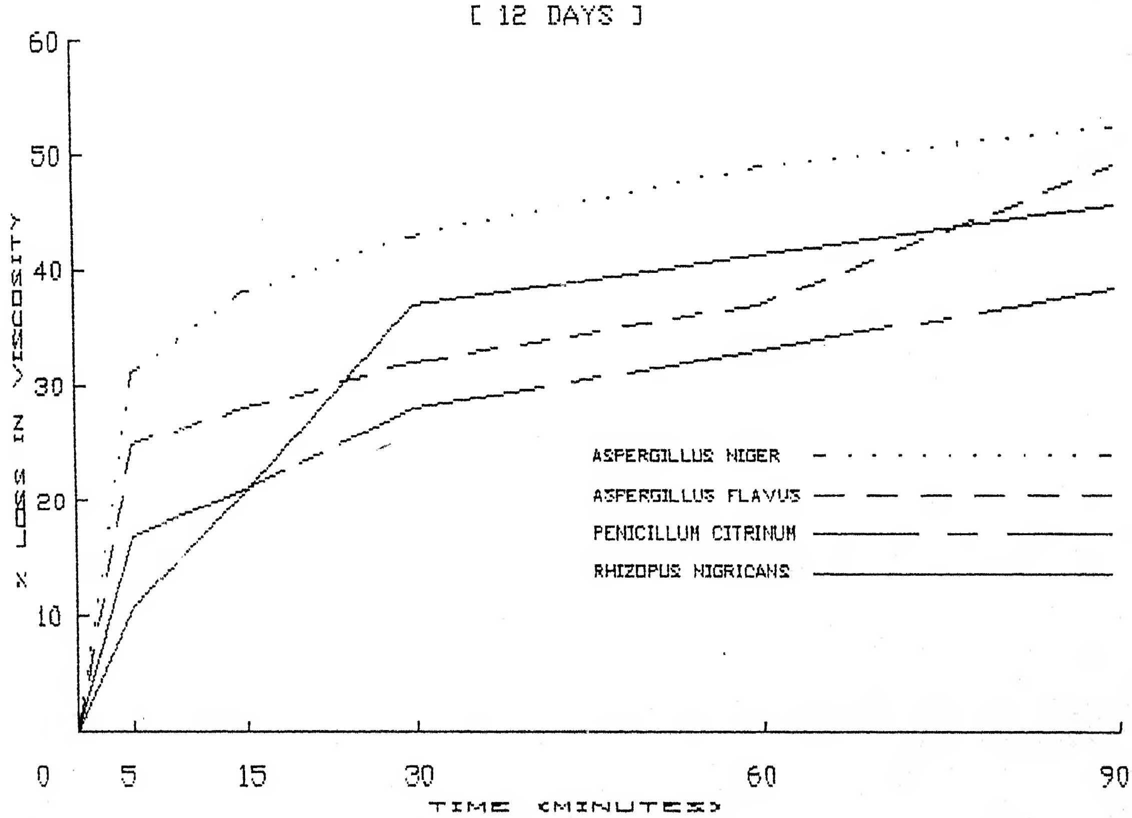
[6 DAYS]



(FIGURE 20 'A')

PMTE (PECTION METHYL TRANSELIMINASE) ACTIVITY AT DIFFERENT INCUBATION PERIOD IN GLUCOSE ASPARAGIN MEDIUM

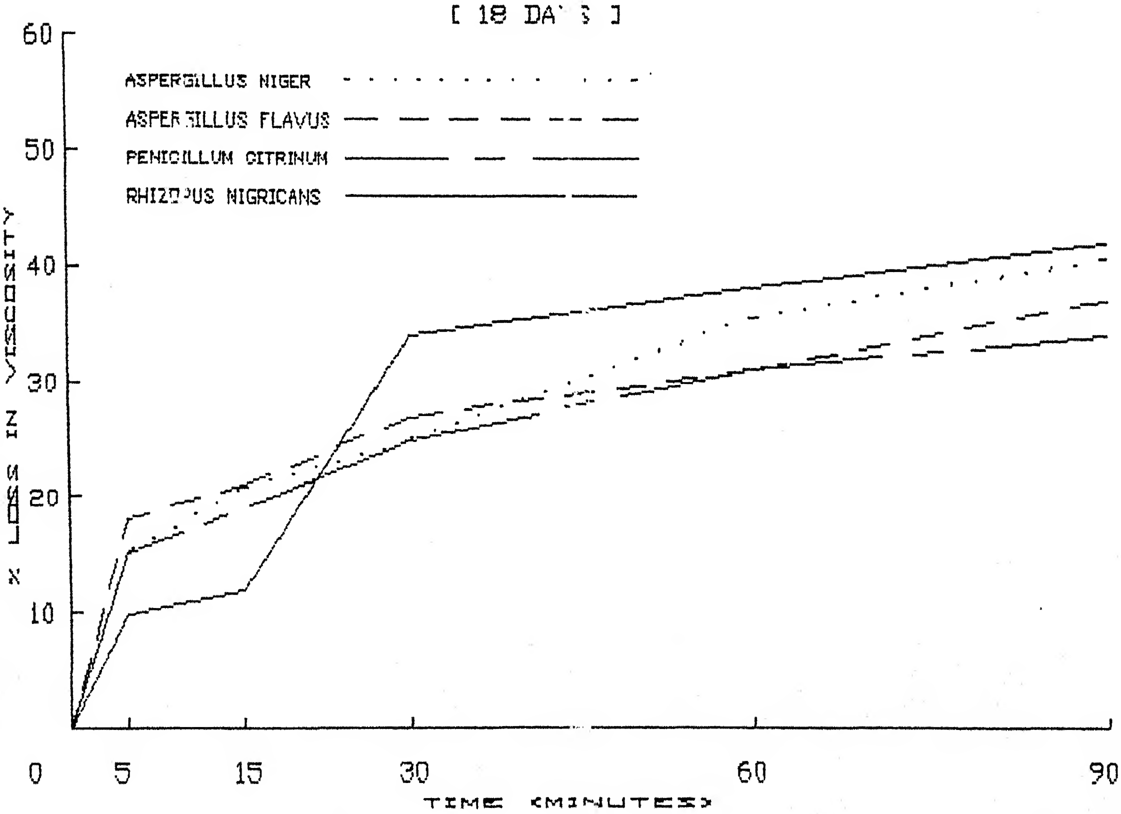
[12 DAYS]



(FIGURE 20 'B')

PMTE (PECTION METHYL TRANSEL.'MINASE) ACTIVITY AT DIFFERENT INCUBATION PERIOD IN GLUCC SE ASPARAGIN MEDIUM

[18 DA' S]



(FIGURE 20 'C)

The amount of activity was quite variable in various fungal organism and also varied according to a incubation period.

Maximum activity of PMG was observed in the medium with Rhizopus nigricans and Aspergillus niger, R. nigricans produced maximum PMG after 6 days of incubation where it gave the REA value of 11.6 units. While A. niger produced maximum PMG after 12 days of incubation i.e. REA 8.6 Units. The rest two test organisms also gave PMG activity but was found to be favoure most, after 12 days of incubation period in A. flavus and after 6 days and 18 days of incubation period in P. citrinum their REA values were equal to 7.8 and 6.25 respectively.

With regard to production of PMTE it was observed that both the sp. of Aspergillus produces maximum amount of PMTE on glucose asparagin medium and that too after 12 days of incubation, their REA values were 11.6 and 10.9 respectively. This was followed by R.nigricans and P. citrinum with REA value 10 and 7.5 respectively. Table XXXV presents the result of PMTE enzyme produced by the test organism. It is clear from the above table the amount of PMTE produced is variable with refrence to the fungal organism. All organisms produced good activity after 12 days incubation period thus it is the optinum period for PMTE production for

all the test organism.

On comparing the trend for the production of PMG and PMTE the results obtained apper to be variable with reference to a test organisms. The behaviour of **A. niger** and **A. flavus** appear to be quite similar, both organisms produced the minimum above refered pectic enzyme after 6 days of incubation period, maximum after 12 days and slightly reduced after 18 days of incubation. **P. citrinum** behaved differently for the production of PMG and PMTE. It gave maximum PMG after 6 and 18 days of incubation and reduced value after 12 days of incubation while for PMTE production maximum production was obtained after 12 days followed by 18 days and minimum after 6 days. Trend, shown by **R. nigricans** for the production of PMG and PMTE was different to **Penicillium** in the sense that it produced maximum PMG after 6 days of incubation and minimum after 18 days of incubation. The PMTE production was maximum after 12 days of incubation period followed by 18 days and 6 days incubation period.

EXPERMENT 'II' :EFFECT OF FUNGICIDES ON THE PRODUCTION OF PECTOLYTIC ENZYMES.

The effect of fungicides on the production of pectolytic enzyme by the test fungus are shown in the table XXXVI and XXXVII.

TABLE - XXXVI

Effect of fungicides on PMG production of fungal test organism

Organisms	Fungicides used	Conc. of fungicide	6 Days % Loss in viscosity					6 Days REA	12 Days % Loss in viscosity					12 Days REA	18 Days % Loss in viscosity					18 Days REA
			5	15	30	60	90		5	15	30	60	90		5	15	30	60	90	
Aspergillus niger	Bavistin	.010%	0	0	0	1	3	0.6	0	0	2	5	8	1.7	0	0	1	3	5	1.1
	Captan	.010%	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
	Aureo- -fungin	10 ugm/ml	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Aspergillus flavus	Bavistin	.010%	0	0	2	3	5	1.1	0	1	3	5	7	1.5	0	2	3	5	7	1.5
	Captan	.010%	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
	Aureo- -fungin	10 ugm/ml	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Penicillum citrinum	Bavistin	.010%	0	0	0	2	4	0.8	0	0	2	3	6	1.3	0	0	2	2	5	1.1
	Captan	.010%	-	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
	Aureo- -fungin	10 ugm/ml	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Rhizopus nigricans	Bavistin	.010%	11.8	12.1	22.8	29	36	8	9	19	30	38	42	9.2	8	11	24	31	38	8.4
	Captan	.010%	8.4	4.4	19	25	32.5	7.1	7.4	9.4	21	24	34	7.5	6.4	10	20	23	30.4	6.6
	Aureo- -fungin	10 ugm/ml	0	1	3	5	8	1.7	1	5	10	15	18	4	2	6	7	11	18	4

- ----> Complete inhibition

TABLE - XXXVII

Effect of fungicides on PMTE production of fungal test organism

S. No.	Organisms	Fungicides used	Conc. of fungicide	6 Days						12 Days						18 Days					
				% Loss in viscosity					REA	% Loss in viscosit					REA	% Loss in viscosity					REA
				5	15	30	60	90		5	15	30	60	90		5	15	30	60	90	
1	Aspergillus niger	Bavistin	.010%	0	0	0	2	2	0.4	0	0	1	3	6	1.3	0	0	0	2	3	0.6
		Captan	.010%	-	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-
		Aureo--fungin	10 ugm/ml	-	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-
2	Aspergillus flavus	Bavistin	.010%	0	0	0	1	3	0.6	0	0	3	5	7	1.5	0	1	2	3	5	1.1
		Captan	.010%	-	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-
		Aureo--fungin	10 ugm/ml	-	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-
3	Penicillum citrinum	Bavistin	.010%	0	0	1.2	1.8	3	0.6	0	0	1	2	5	1.1	0	0	1	1	4	0.8
		Captan	.010%	..	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-
		Aureo--fungin	10 ugm/ml	-	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-
	Rhizopus nigricans	Bavistin	.010%	10.8	16	19.6	30	34.	7.5	12.8	18.1	20	31	38.	8.4	7	14	20	26	34	7.5
		Captan	.010%	9.6	10	15.4	18.9	20	4.4	7	8	18	20	29	6.4	8	9	18	20	27	6.02
		Aureo--fungin	10 ugm/ml	0	1	3	4	6	1.3	3	4	9	11	15	3.3	3	5	7	9	11	2.4

- ---> Complete inhhibition

It is clear from the data that all fungicides caused a varing amount of inhibitory effect on PMG and PMTE.Captan and Aureofungin were most severe in causing inhibitory effect where the growth of A. niger, A. flavus and P.citrinum was completely inhibited under the concentration used thus low enzyme activity could be recorded. R.nigricans showed less toxic effect for the synthesis of these enzymes. Aureofungin however considerably reduced the production of these enzymes. A perusal of table XXXVI and XXXVII that inhibitory effect was most severe on PMTE production as compared to PMG. A.niger, A. flavus and P. citrinum showed some enzyme production under the influence of Bavistin, but as compared to the production of these enzymes without fungicides this enzyume activity is negligible. Bavistin and Captan were comparatively less effective for this purpose against R. nigricans. The observation recorded during the experiments on radial growth and mycelial mat production. R.nigricans showed that the vegetative growth of this organism was not influenced by either Bavistin or Captan. Aureofungin however reduced the vegetative growth but that too was very little as compared to control. From the studies conducted in this experiment it is clear that these fungicides had definietely reduced the pectic enzyme production of R.nigricans and there is no correlation between

vegetative growth and the enzyme production., These observations again however confirmed the inhibitory effect noticed in the previous experiment on spore germination.

CHEPTER -21

SUMMARY & DISCUSSION

Cellulolytic and pectolytic enzyme production by the selected fungal test organims were determined in this section. The results obtained are shown in the table XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI & XXVII & fig. 17 A. B. C. & 18 A. B. C.

The enzyme activity was studied using standard viscometric procedures for cellulose (cx) enzyme production and for (PMG and PMTE) pectolytic enzyme production.

The following is the summary & discussion to which the author arrived.

Part I - Studies on cellulolytic enzyme.

To determine the optimum cultural conditions for the production of cellulolytic enzyme by **Aspergillus niger**, **A. flavus**, **Pencillium citrinum** & **Rhizopus nigricans**, the organisms were grown on glucose asparagine & basal medium with cotton as the sole carbon source. Age of culture was also studied where three incubation periods were considered i.e. after 6, 12 and 18 days. It was observed that glucose asparagin medium was more favourable for the production of cellulase enzyme. Secretion of enzyme was also effected by different incubation periods. On the basis of over all results it may be said that glucose asparagine

medium with 6 days incubation periods showed good performance for A.niger, A. flavus and R. nigricans, while P. citrinum showed good performance on the same medium after 12 and 18 days of incubation period.

Effect of fungicides on production of cellulolytic enzyme. The secretion of cellulase and mycelial growth of A.niger, A. flavus and P.citrinum and R. nigricans was strongly suppressed by certain fungicides. Among these Captan and Aureofungin were most effective and Bavistin was less toxic, it could develop a very poor inhibitory effect of cellulase formation in R.nigricans. As compared to Captan and Aureofungin, Bavistin was again least effective against A. niger, A. flavus and P. citrinum though the enzyme production was noticed but their values were almost insignifacant as compared to that of control. The mycelial growth of Aspergillus and Penicillium was less as compared to R. nigricans. Specially A. flavus, where very little mycelial production could be noted but the enzymatic activity obtained was quite high. Thus it appears that there can be no correlation between mycelial growth and enzyme production. Similar observations were obtained by Sindu and Sandu (1979).

The presence of cellulase activity in cultures filtrates of P. citrinum was quite good, on the 6th day incubation specially when basal medium was

taken. Presence of its activity on the 2nd day on absorbant cotton cultured with **Penicillium expansun** was also noticed by Daniel & Dube (1983); Mehta (1974) had recorded the inhibitory effect of fungicide on cellulase production. These observations supports the observations made by the author.

Part II :- Studies on pectolytic enzymes.

The production of pectic enzymes by microorganisms is also influenced by cultural conditions, incubation period and by the presence of fungicides. Therefore to determine the effect of cultural conditions, incubation period and fungicides, the present studies were made and results obtained in the table XXXIV, XXV, XXXVI, XXXVII and Fig. 19 & 20 are being discussed as below.

Effect of age of culture on pectic enzyme productiuon.

The test organism **A. niger**, **A. flavus**, **P. citrinum** and **R. nigricans** produced varying amount of PMG & PMTE in different incubation periods. PMTE production was found to be most favorable after 12 days incubation period than 18 days or 6 days incubation periods for all the test organisms, studied. 6 days incubation period was found to be least favorable for PMTE production.

Regarding the effect of PMG production it was found that the best PMG production was obtained in both the <u>Aspergillus sp.</u> after 12 days of incubation period and for <u>P. citrinum</u> and <u>R. nigricans</u> after 6 days of incubation periods . It may also be pointed out here that in <u>R. nigricans</u> as the incubation period increased the PMG production decreased while in <u>P. citrinum</u> the PMG poduction remained almost the same after different incubation period studied. On the basis of overall results it may be stated conclusively that <u>A. flavus</u>, <u>A. niger</u> and <u>P. citrinum</u> appears to be more efficient for the production of transeliminases than glycosidases, however <u>R.nigricans</u> appears to be more efficient for the production of glycosidases than transeliminases.

The results obtained also indicate that there could be no correlation between fungal growth and enzyme production because in certain cases it was found that there was more mycelial production and less enzyme production.

<u>Effect of fungicides on pectic enzyme production</u> :-

The effect of fungicides on pectic enzyme production are shown in the table XXXVI and XXXVII.

All the three fungicides used in the present study showed variable effect on the secretions of pectic

enzyme and fungal growth of A. niger, A. flavus , P. citrinum and R. nigricans, The formation of PMG enzyme in all the test fungi were less strongly suppressed as compared to the PMTE activity. Among these however Captan and Aureofungin were most effective against all the test organisms. Bavistin,also considerably reduced the enzyme production causing maximum inhibitory effect on A. niger, A. flavus, and P.citrinum while less effective on R.nigricans. Considerable inhibition of fungus growth was caused by these fungicides. No growth at all was obtained in presence of Captan and Aureofungin of A. niger, A. flavus and P. citrinum. Aureofungin however reduced growth in R. nigricans but Bavistin and Captan appear to be not at all effective on the mycelial growth. There appeared to be no clearcut correlation between secretion of enzyme and fungal growth in precence of these fungitoxic substances.

Inhibition of pectic enzyme production by various fungicides in several fungal organisms has also been reported by Grover (1964).

The fungal organisms A. niger, A. flavus, P. citrinum and R. nigricans were found to be having the best percentage frequency of occurance during the studies on islolation . In this section it was observed that these organism are also capable of producing

pectolytic and cellulolytic enzymes, their growth, germination of spores and enzyme producing capability could inhibited by using the fungicides in minimum inhibitory concentrations. The fungicides used by the author were Aureofungin Bavistin and Captan. The ability of fungicides to control plant diseases and inhibit the production and activity of cell wall degrading enzymes has also been demonstrated by number of workers including Grover (1964); Goel and Mehrotra(1973), Mehta (1974). Thus this property of these fungicides can be exploited for controlling or inhibiting the fungal organisms showing dynamism on cotton. This would indirectly help in preventing the pathological or allergic responses produced by these and other organisms, which are incorporated or present on cotton fibres floating or lying inside the cotton mill.

SECTION V

GENERAL SUMMARY AND CONCLUSION

CHAPTER - 22

GENERAL SUMMARY & CONCLUSIONS

Jhansi, situated at a latitude of 25 degree. 27' N and at a longitude of 38 degree. 35' E, has typical monsoonic climate which can be divided into three distinct seasons, i.e. rainy, winter & summer. Rainy season is from mid June to mid October, winter season is from mid October to mid February & summer season is from mid February to mid June. Wind acquires maximum speed during summer and rainy seasons, while it is mild in winter season.

U. P. State spinning mill the, place of which exploration was done is situated at Jhansi - Gwailor road near the air field. It has two working units with about five thousand workers (in the age group 18 - 50 years) involved in day & night shifts.

The workers here complains for various health problems like cough, chest and abdominal pains, Asthma etc. These and many other respiratory disorders have also been reffered to develop on mill workers by Gupta & Gupta (1984). Most of these workers belongs to a very low socio - economic status of eastern U. P.. These may have some pre existing pathological infestations, involving the pulmonary track, skin & other organs of the body, thus may be the carriers of such disease producing organisms. Because of the

inadequacy of medicinal aids and nutritional deffiaiences they may herbour such organisms for a very long times. Thus the microorganisms inhabiting cotton & those incomporated during handling are concerned with serious hazards. This has prompted the author for the present investigations. The investigations conducted are being summarised below.

MICROBIAL DYNAMISM.

Cotton bales that arrive are kept in store for being processed in the two working units of mill. Each unit has an underground absorbing channel in which the lint and dust floating in air is being constantly trapped under suction. The cotton is being spun into threads in three chambers of each units where temperature and humidity is being maintained. To enumerate the microbial dynamism operating on cotton fibres periodic collection of cotton samples was done. For convenince three sites where decided from where cotton samples were picked up regularly at an interval of fifteen days for one year and isolation of microbial population was done from them. In addition to these cotton samples airospora survey, for microbial population operating on fibres floating in air, inside and outside the mill was also done at the same time.

These constituted the two additional sites. Thus quantitative and qualitative analysis of microorganisms obtained from the above five sites were done.

Fungi were grown on Potato dextrose agar (Johnson et.al., 1959) and Peptone dextrose agar with rose bengal & streptomycin (Martin 1950). Bacteria were grown on Soil extract agar (Allen 1957) , Nutrient agar (Waksman 1961), Blood agar (Cruickshank et. al., 1972), Lowenstein and Jensen medium (Jensen 1961).

Identifications were done using standard taxonomic keys and monographs reffered in the Appendix II and confirmed them from the identifications arrived from "Kew" England.

Absolute number of oganisms per gram dry weight and percentage occurrance obtained from site I i.e. absorbing channel, site II i.e. store room and site III i.e. spinning chambers are given in table III to VIII and Fig. 3 to 8. From these datas it will be evident that the highest absolute number of organism per gram dry wt. of cotton was obtained from site I. At this site the cotton fibre get mixed up with dust & other organic matter. It remains here for a considerable long period while at site II & III fibres are being constantly picked up renewed & comparatively less

incorporated with dust. Thus flaring up of organism in percentage and absolute number at site I must be due to this very reason. From the over all results obtained at these sites it was found that the absolute number, of bacteria occured in the range of 866.7×10^{5} /gm. dry wt. of cotton to 3.0×10^{5} /gm dry wt. of cotton and of fungi occurred in the range of 85.5×10^{5} to 0.08×10^{5} /gm. dry wt. of cotton. The absolute number of microorganisms fluctuated while sampling , during the entire period of study. During this period they reached the peak level 2 - 3 times alternating with the decline, this might be due to the type of organic matter being consumed coupled with the effect of climatic factors. Some workers has reffered the initial rise due to the flaring up of primary moulds the second peak due to the secondary moulds and the third peak due to the tertiory moulds which are more or less stable (Garrett 1951; Bharat Rai 1970; and Kaarink 1974). However the author could not distinguish between primary, secondary or tertiory moulds. This observation is alike to the one observed by Mehrotra and Aneja (1979); Kamal and Shrivastava (1975).

At site 4 and 5 i.e. the airospora inside and outside the spinning mill exploration was done for the average number of organisms per plate. The data

obtained are given in the table IX to XII. The number of forms outside the mill were comparativily larger, as compared to the number of forms inside the mill. This might be due to the more climatic variations outside the mill and also due to the involvement of more components of the ecosystem of the outer atmosphere.

Qualitatively 24 species of fungi belonging to 13 genera were observed. The monthly seasonal variation of different fungi isolated has been shown in table XIII. The data recorded for the percentage occurrence of different fungi from various sites shows that almost the same fungal organisms occurred in high percentage, while some made occasional appearance. This might be due to the poor colonizing ability of these organisms. Many species during isolation showed rapid multiplicaton at first, dwindled as the environment changed. It appeares that temperature and change in the availability of food probably has a greater influence in determining the species of the organism comprising the populaton at any one time.

The percentage frequency of fungal organisms appeared at various sites are given in the table XVII. A persual of this table shows that **Aspergillus niger** was found in highest percentage occurrence at all the sites followed by **A. flavus**, **Penicillium citrinum** and

Rhizopus nigricans. These organisms contributed the bulk of microbial population of every isolation. Thus these organisms were selected for further investigations. In addition to these fungal organisms some bacterial organisms were also frequently observed on isolation plates. These were Staphylococcus aureus, Streptococcus pyogenes, Mycobacterium tuberculosis, Bacillus Sp. and Proteus Sp. Since these are involved in several human pathogenic complications these were also selected for the studies on control measures.

In the subsection 'B' of this section the above four fungal organisms were subjected to their studies on cellulolytic abilities. Datas obtained are recorded in the table XVIII. Among these organisms Aspergillus niger demonstrated the maximum cellulolytic ability resulting in 22% loss in the dry weight of cotton. This was followed by A. flavus with 12.6% P. citrinum 10.8% and R. nigricans 11% loss in the dry weight of cotton. The observations recorded point towords their affinity for cotton and the good performance justify their high percentage frequency obtaind during isolations.

Data

After determining the cellulolytic ability of the selected fungi, their competitive saprophytic colonization was studied in the sub section 'C' of this

section. The datas are recorded in the table XIX and fig. 13 to 16. The observed results show that these organisms are quite successful saprophytic colonizers. The growth of all the four test fungus was reduced when sample water suspension was inoculated and incubated for 24 hrs. before inoculating the test fungus. It was also observed that the test organisms showed easy colonization in the begining but after 24 hrs. they showed depression in their growth rate. The sample water suspension obtained from cotton picked from absorbing channel and that from the store room gave the same results.

CONTROL MEASURES

Aspergillus niger, *A. flavus*, *P.citrinum* and *R. nigricans*, performed good cellulolytic ability and also demonstrated siginificant competitive saprophytic colonization capabilities during the studies conducted in the previous section. These performances justify their highest percentage occurrance obtained during isolation experiments.

Apart from the above these organisms are often associated with diseases like automycosis, pulmonary "fungus balls", eczematoid lessions, open ulsers, mucor -mycosis etc. Their afflotoxins are also reported to have a direct toxicity and long term carcinozenic

effect on animal tissues (Rati & Ramalingan 1979; Diener et. al., 1976; Rashmi Tewari 1991).

To over come the hazard to human health that may result from such fungal organisms and the bacterial organism reffered above the experiments on control measures were planned and worked out in this section. Easily available and well known fungicides & antibiotics were employed prevent the growth and activity of such organisms.

During the preliminery test fungicides and antibiotics were screened against the above fungal organisms fungicides were used in .5% concentration, while antibiotics in .25 ugm/ml concentration. The results are given in the table XX. From this test Captan, Bavistin and Aureofungin were selected on the basis of their performance none of the antibiotic could inhibited the growth of any of the test fungus. Out of the 8 antibiotic tested in the above concentration against bacterial test organisms 7 gave positive results. Their results are given in the table XXII.

After selecting the fungicides and antibiotics from the preliminary test further studies were conducted to explore the posibility of an overall

control measures. For further test fungicies were used against fungal organisms and antibiotics were used against the bacterial organisms because of their performance towards the respective groups of organisms in the above experiment.

In the next experiment studies were conducted to get the minium inhibitory concentrations of fungicides and antibiotics. Fungicides used aginst fungi were Captan, Bavistin in. 020%, .010% and .005% concentrations and Aureofungin in 1, 5, 10, and 15 ugm/ml concentration. The results obtained are given in the table XXIII. The observations were made for their effect in causing inhibitory effect on the radial growth ofthe test organism. The lowest concentration in the series of dilution having no growth after 48hrs. was taken as M.I.C. for that fungicides. **Rhizopus nigricans** was found to be the most tolerent fungal organism. Neither Bavistin nor Captan was able to inhibit this organism at these concentrations. Aureofungin gave some inhibitory effect at 10 ugm/ml concentration and at 15 ugm/ml concentration inhibited the growth of **R.nigricans** completely. This observation of the author is quite similar to the one found by Thakre and Johri (1973). Against the rest of the three fungal organisams i.e. **Aspergillus niger**, **A.flavus**, and **P. citrinum** these fugicides were quite active. Captan

and Bivistin recorded their M.I.C. at .010% concentration while Aureofungin at 10 ugm/ml concentration.

Against bacterial organisms Penicillin, Streptomycin, Tetracyclin, Erythromycin, and Rifamycin were used. Each antibiotic was developed in a series of 15 dilutions i.e. .002, .005, .012, .025, .05, .075, .1, .15, .2, .3, .4, .5, .6, .9 and 1.0 ugm/ml of the medium. The results obtained are given in the table XXIV A and in the plates 4, 5 and 6. M.I.C. of the above antibiotics obtained against Staphylococcus aureus, Streptococcus pyogenes and Bacillus sp. are as follows. Erythromycin .6, .05 and .4 ugm/ml, Penicillin .02, .1 and .02 ugm/ml, Rifamycin .005, .05 and .075 ugm/ml; Streptomycin .9, .9 and .6 ugm/ml, Tetracyclin .1, .05 and .1 ugm/ml concentrations respectively. Proteus sp. was not at all inhibited in any of the above dilution of all the antibiotic tested. Mycobacterium tuberculosis in the above dilutions too was not inhibited by penicillin and Erythromycin.

Against M.tuberculosis Rifamycin recorded its M.I.C. at .005 ugm/ml, Streptomycin at .5 ugm/ml. Thus against Proteus sp. and M. tuberculosis in another experiment raised concentration of those antibiotic were used which could not inhibit the organism in the

above dilutions. The series of dilution prepared for this test consisted of 10, 15, 30, 45, 60, 70, 90, 100 and 200 ugm/ml concentrations. The results obtained are given in the table XXIV B. In this experiment the M.I.C. recorded of various antibiotic against <u>**Proteus sp.**</u> is as follows. Erythromycin at 90 ugm/ml, Penicillin at 200 ugm/ml, Rifamycin at 30 ugm/ml, Streptomycin and Tetracyclin at 100 ugm/ml concentration. Since except that of Rifamycin these concentration are very high and could not be used because of their toxicity in blood, they are considered to be uneffective. <u>**Proteus sp.**</u> is thus regarded as resistant towards these antibiotics except Rifamycin where the sensitivity was found to be 30 ugm/ml concentration. The conclusions drawn by the author from these results are similar to the one made by John W.corcom and Fred (1969). Against <u>**Mycobacterium tuberculosis**</u> the M.I.C. of Penicillin and Erythromycin were found to be 15 and 10 ugm/ml respectively. The M.I.C. obtained of the above antimicrobial agent are collectively given in the Table XXV.

The above results were confirmed later in an another experiment in which the radial growth of the organisms were observed on media under the influence of the above fungicides / antibiotices. The results obtained are given in the table XXVI. In this

experiment the radial growth of fungal organisims were observed on agar media having Bavistin and Captan in .020% , .010% and .005% concentrations while Auerofungin in 15 , 10 and 5 ugm/ml concentration. Captan was found to be most effective followed by Bavistin and Aureofungin for **A. niger** , **A. flavus** and **P. citrinum**. Against **Rhizopus nigricans** moderate inhibition was recorded at 10 ugm/ml concentration while on 15 ugm/ml concentration the results was quite satisfactory. Bacterial test organisms were observed on agar media having antibiotics in 3 dilutions. Dilutions selected were in relation to the results and observations made during the experiment to obtain the M.I.C. All the 5 antibiotics used i.e. Erythromycin, Penicillin, Rifamycin, Streptomycin and Tetracyclin significantly inhibited the growth of the test organisms except **Proteus sp** whcih grew almost unaffected. From the over all results obtained it could be said that the growth of the test organisms was gradually inhibited with a progressive increase in the concentration of the antimicrobial agent. In lower concentrations a reverse trend was observed with increase in incubation period from 0 hr. to 72 hrs.

After observing the effect of fungicides against the fungal organisms on solid media it was considered appropriate to have an idia of its effect on mycelial

growth and dry weight in liquid medium. Bacteria were not included in this experiment since (1) they do not produce mycelial growth of any significant and (2) their growth in liquid media was already studied. Their effect on population and spore germination has been taken up in the next experiment. In this experiment Bavistin and Captan were used in .030%, .020% and .010% concentraton while Aureofungin was used in 10, 5 and 1 ugm/ml concentration. The results obtained are given in the table XXVIII. In this experiment the results obtained aginst **R.nigricans** gave some clue for understanding the antimicrobial behaviour of the fungicides used against this organism. In this experiment these fungicides, which were observed to be uneffective during the previous experiment, showed inhibitory effect when **R.nigricans** produced less mycelial weight then that of control under their influence. **A. niger**, **A. flavus** and **P. citrinum** was completely inhibited by Captan in all dilutions tested and by Bavistin at .030% concentration. The slight mycelial growth of above fungal organisms recorded in this experiment under the influence of Bavistin might be due to its lesser toxicity and secondly the inoculum disc floated on liquid media while inocubation might be instrumental in producing such growth. On soild media the aerial grwoth which occurred on the inoculum disc never developed on agar medium below

beyond the 7mm inoculum disc thus its growth was not recorded in the table but in this experiment the mycelial weight was observed since the mycelial weight which appeared on the inoculum disc had to be included while calculating the total weight obtained.

After the above experiment final experiment was plannned and conducted to study the effect of fungicides and antibiotic on spore germination of test organism. Datas are recorded in the table XXIX and XXX. The spore germination of fungal organisms were studied microscopically at 4, 8 and 12 hrs. interval concentration used were the same as used in the radial growth. In this experiment it was observed that the swelling of spore and their germination are two distinct phase, of a common but complex phenomenon. The former was less sensitive to the action of fungicides than the latter. In general though verying degrees of inhibitory effect were observed but as the incubation period increased the rate of germination decreased. **Penicillium citrinum** showed slow spore germination, even in control experiment it took 24hrs to germinate. **R. nigricans** appear comparatively resistance to the action of fungicides. These observation of the author are similar to that of Thakre and Johri (1973-74).

Effect of antibiotic on spore germination of bacterial organism were studied following the method of

Queshel et. al., 1971 and data were recorded in table XXX. Here also the same dilution were used as in radial growth. These observation once again confirm the M.I.C. obtained in previous experiments. Except **Proteus sp.** against the rest of bacterial organisms concentration of 5 antibiotics recorded for total inhibition of spore germination was below 1.2 ugm/ml **Mycobacterium tuberculosis** showed very slow growth and thus its activity on radial growth and spore genrmination could not be obeserved. It's growth is so slow that the culture does not become, macroscopically visiable until 10 to 14 days or some time as late os 6 to 8 weeks. And thus it was excluded because this delayed grwoth may cause a false impression of an inhibitory effect.

ENGYMOLOGICAL STUDIES

Cellulolytic and pectolytic enzyne production of the selected fungal organism were determined in this section. The results are shown in the tables XXXI to XXXVII. Standard viscomitric producere were adopted for cellulase (Cx) enzyme production and pectolytic (PMG & PMTE) enzyme production.

During the studies on cellulolytic enzyme glucose asparagine medium was found to be better for cellulose production of **A. niger**, **A. flavus**, **P. citrinum** and **R. nigricans.** Among the incubation period tested 6

days incubation period was found to be better for enzyme production of A. niger, A. flavus and R. nigricans. While P. citrinum gave better production after 12 to 18 days incubation. Among the fungicides tested for their inhibitry effect on cellulose production, Captan and Aureofungin were found to most effective and Bivistin less toxic. It was observed that the mycelial growth had not corelation with the amount of enzyme produced. Among the fungal organisms tested A. niger gave best cellulolytic ability followed by A. flavus, R. nigricans and P. citrinum .

The data recorde for the studies on pectolytic enzymes are given in the table XXXIV to XXXVII.

The test organisms produce variable amount PMG and PMTE in different incubation period. PMTE production was most favarable 12 days of incubation for all test fungal organism regarding PMG production both Aspergillus sp. gave the best production after 12 days of incubation while P. citrinum and R. nigricans gave better production after 6 days of incubation. Here also fungal growth could not be corelated with the enzyme production.

In the experiment to determine the effect of fungicides on pectic enzyme production it was found

that PMT production was less strongly suppresed as compare to PMTE activity. Amongs the fungicides tested Captan and Aureofungin were most effective against all test organisms followed by Bivistin. Bivistin though reduced the enzyme production of all the test fungal organisms but it caused miximum inhibitory effect on A. niger, A. flavus and P. citrinum and less on R. nigricans.

From the overall results it could be concluded that the fungal organisms normaly Aspergillus niger, A. flavus, Penicillium citrinum and Rhizopus nigricans have proved to be good colonizers of cotton, they have good competitive saprophytic ability and at the same time are having effecient enzyme producing capabilities and thus could cause a good deal of cotton deterioration if allowed to remain there for a considerable period. Their presence should not only be inhibited because of their cotton decomposing capabilities but also because of their involvement in several pathological infestations. Specially at localities and places which involve human population in such a large number. Together with the fungal organisms the bacterial organisms to which the author came across are also of not less significant. Their growth and develpment should also be prevented. For this the results obtained during the control experiment may be

implemented. Captan and Bivistin be recommended at .020% concentration and Aureofungin at 15 ugm/ml. concentration. While any of the 5 antibiotics used normaly Penicillin, Streptomycin, Tetracyclin, Erythromycin and Rifamycin could be recommended in 1.2 ugm/ml concentration. The last three of the antibiotic could be safely used because, they have no side effect or allergic responses. These antimicrobial substance could be used in the said concentration for periodic spray but before making recommendation for the regular periodic spray of the fungicides their allergic responses should also be investigated.

SECTION VI

MISCELLANEOUS

BIBLIOGRAPY

ABDEL, KADER, M.I.A.,H.N.Abdel- Fattah and S.Hamida (1981). Antifungal effects of Bavistin, corotan and curacron when incorporated with Agar or liquid medium, Pak. J. Sci. Res. 33 (1-4): 57-63.

AGARWAL, S. C.(1969). Studies on litter decomposition by some litter fungi, Philline. J.Sci., 98 : 303 - 311.

AGARWAL, S. C.(1971). The cellulolytic capacity of some forest litter fungi. Phyton. 23(2):169-179.

AGARWAL, S. C.,D. P. C. RAO. (1974). Effect of some antibiotics on the mycelial growth and on the production of amylase by some Keratinophilic fungi. Hindustan antibiotics. 16 (4) : 206 - 209.

AGARWAL, R. K. & M.J.Thirumalachar, (1966). Effect of aureofungin on powdary mildew of apple. Ist Inter symp. Plant pathol. Dec. 27, Dehli, Abstr. of papers. Indian Phytopathological society. 52.

AHMAD, N. and A.N.Gulatia. (1943). The effect of storage under certain specified conditions on the quality of Indian cotton. Ind. J.Agric Sci. 13:494-514.

ALEXANDER, M.(1961). "Introduction to soil microbiology "John wiley & son's , New York. 472.

ALLEN, I.Laskin. (1968). Tetracyclin antibiotic mechanism of action. PP 331. springer verley Berlin, neideal berg, New York.

ADERSON, J. G. & J.E.Smith (1971). Synchronus initiation & maturation of Aspergillus niger condiophores in culture. Trans, Br. Mycol. Soc.56:9-29.

ANON, (1943) Defination of fungicidal terms, Phytopathology. 33:624-626.

ASHWORTH, L. J , J. L. Mc. Mean and C.M.Brown. (1969). Infection of cotton by Aspergillus flavus, time of infection and the influence of fibre moisture. phytopathology, 59(3):383-385.

ASHWORTH, et. al. (1971). Phytopathology. (5):488-493.

AYERS, W. A , G. C. papvizas & K.P.Link. (1966). Phytopathvarious. 56 : 1006 - 1011.

BATEMAN, D. F. (1964). Cellulase & the Rhizoctonia disease by bean. Phytopath. 54.(11):1372-1377.

BATEMAN, D. F. (1966). Hydrolytic & transeliminative degradation of pectic substance extracelluar enzyme of Fusarium solani, F. phaseoli. Phytopathology. 56: 238.

BATEMAN, D. F. & R. L. Millar. (1966).Pectic enzyme in tissue degradation, Annl, Rev. of Phytopathology. 4:119.138.

BATEMAN, D. F. (1969). Some characteristics of cellulase systen produced by Sclerotium rolfsic, Scace. Phytopath. 55 (2): 204-211.

BAGGA, H. S. (1970). Pathogenicity studies of organism involved in cottton boll rot complex. Phytopathology. 60(1):158-159.

BELLAMY, W. D. (1974). Single cell protiens from cellulosic waste. Biotechnol. Bweng: 16:869-880.

BHATIA, D. S. & J.S.Chohan. (1970). Production of Polygalacturonase by Aspergillus niger in vitro & viva. Indian Journal of microbiology. 10 (1).

BLAIR, I. D. (1943). Behaviour of the fungus Rizoctonia solani, Kuhn, in Soil . Ann. Appl. Biol . 30:118-127.

BLUM, R. & W. H. Stahl. (1952). Enzymatic degradation of cellulose, fibres. Textile Resh. J.22:178-192.

BRIERLEY, W. B., S. T. Jewson & M. Brierley, (1928). The quantitative study of soil fungi. Proc. Ist Int. Congr. Soil sci. Com. IV:48-71.

BROWN, W. (1915). The action of Botrytis cinerea Ann. Bot. 29: 315-348.

BUTLER, F. C. (1953a). Saprophytic behaviour of some cereal root rot fungi. I saprophytic colonization of wheat straw . Ann. Appl. Biol. 40: 284-297.

BUTLER, F. C. (1953b). Saprophytic behviour of some creal root rot fungi II Factors influencing saprophytic colonization of wheat straw. Ann. Appl. Bio. 40: 298-304.

BUTLER, F. C. (1953C). Saprophytic behaviour of some cereal root rot fungi. III. Saprophytic survival in wheat straw buries in soil. Ann. Appl. Biol,. 40: 305-311.

CAPPELLINI, R. A. (1966). Growth & polygalacturonase production by Rhizopus stolonifer. Phytopathology. 56: 734-737.

CHAPMAN, E. S., E. Evans, M. C. Jocobelli & A. A. Logan. (1975). The cellulolytic & amylolytic activity of populaspora thermophille. Mycologia. 67:608-615.

CHAURASIA, S. C., K. M. Vyas & N. K. Soni. (1973). Effeciency of certain antibiotic against Phytophthora Parasitica var. Piperina causing leaf rot of pan.

CHEEMA, S. S. & R. Jeyarajan, (1971). In vitro effect of Aureofungin on spore germination in Alternaria citri. Hindustan antibiotic Bulletin. 13(3)(4):65-66.

CHESTERS, L. G. C. (1960). Certain problems associated with the decomposition of soil organic matter by fungi. In the Ecology of soil fungi. ed. D. Parkinson & J. S. waid. Liverpool university press.

CHESTER, W. Emmons, Chapman, H. Binford, John P. UTZ., K. J. Kwon - Chung (1977). Medical Microbilogy. Third edition, K. M. Vargnese company Bombay.

COLE, M. A. & A. J. Turgeon. (1978). Microbial activity in soil & litter under lying bandane & calcium arsenate treatment turf grass. Soil Biol. Biochem. 10: 181-186.

COLLINS, R. J. & W. Feldman. (1965). Attempts to improve the growth of Rohopholussimilis infected citrus with undertree drencles of zinephos. Plant Disease rept. 49 : 865-67.

CROWFORD, D. L. & R. L. Crowford. (1976). Microbial degradation of Lignocellulose. The lignin component. Appl.enviro.Microbiol. 31.714-717.

COULTS, A. D. & R. E. Smith (1976). Factors influencing the production of cellulase by Sporotrichum thermophile. Appl, enviuron. Microbiol. 31: 819-825.

DANIEL, D. & H. C Dube. (1983).Cellulase & Glucosidas production by Penicillium expavsin. Indain Phytopathology. 36:738.

DEMIAN, A. L. and H. E. Phaff. (1957). Recent advances in the hydrolysis of pectic substance. Waller stein. Labs communs. 20:119-140.

DIENER, V. L., R. E. Wangener, G. Morgan - Jones & N.D.Davis. (1976). Taxogenic fungi from cotton. Phytopath. 66:514-516.

DOBBS, C. G. and W. H. Hinson. (1953). A widespread fungistasis in Soil. Nature, lond. 172:197-199.

DOMECH, K. H. (1964). Soil fungicides. Ann. Rev. Phytopath. 2: 293-320.

DONALD, D. E. Munneckea. (1972). Factors affecting the effeciency of fungicides in soil. Annl. Rev. of Phytopath. 10 : 375.

DWIVEDI, S. R. and U. P. Singh. (1974). comparative cellulolytic ability of some litter inhabiting fungi. proc. Indian, naton. sci. Acad. Buo. 420-423.

EGOROV, N. S. (1985). concept of antibiotics & their classification. 'Antibiotics' A Scientific approach. 40-41.

ELLIS, M. B. (1971). Dematiaceous Hypomycetes, Kew England common Wealth Mycological Insititute. 608 PP.

ELLIES, M. B. (1976). More Dematiaceous Hypomycetes, common wealth mycological institute, Engaland, Publication . 507 PP.

ERIKSEN, J. and J. Goksoyr. (1976). The effect of temperature on growth & cellulase (B-1, 4 endoglucanase) production in the compost fungus Chaetomium thermophile var dissitum. Arch. Microbiol. 110:233-238.

ERIKSON, K. E. and W. Rzedowski, (1969). Entracellular enzyme system sitilized by the fungus Chrysosporium lignorum for the break down of cellulase. I. Studies on the enzyme production.

FERGUS, C. L. (1964). Thermophilic & thermotolerent mold & actinomycetes of mushroom compost during peak heating. Mycologia. 56.267-284.

________ (1969). The cellulolytic activity of thermophilic fungi & actinomycetes, Mycologia. 61 : 120 KS.

FLETCHER, J. (1969). Morphology & nuclear behaviour of germinating conidia of Penicillium griseofulum. Trans. Br. Mycol. Sec. 53 : 425-432.

FRANKLIN, T. J. & G. A. show (1971). Biochemistry of antimicrobial action, champman & hall Ltd. London. 163.

GARRETT, S. D. (1944). Root disease fungi, Walthmnan. Mass U.S.A. 177.

________ (1950). Ecology of root inhabiting fungi. Bio Rev. 25:220-254.

________ (1951). Ecological groups of soil fungi, a survey of substrate relationship . New phytol. 50:149-166.

________ (1956). Biology ofroot infecting fungi, cambridge university press, London. 293PP.

________ (1962) . Decomposition of cellulase in soil by Rizoctonia solani. Kuhn. Tran, Brit mycol. Soc. 45(1): 115-120.

________ (1963). Saprophytic competition in the soil fungi & soil fertility. Pergamen Press Ltd. London. 115-132.

________ (1963a). A comparision of cellulose decomposing ability in five fungi causing cereal foot rots. Tran. Br. Mycol Soc. 46: 572-576.

________ (1963b), Soil fungi, Soil fertility, Oxford, Pergaman press, 165.

_______ (1966), Cellulose decompsing ability of some creal foot rot fungi in relation to their saprophytic survival. Trans. Br. Mycol. Soc. 49:57-68.

GASCOIGNE, J. A. and M. M. Gascoigine (1960). Biological degradation of cellulase. Butter worths & Co. London. 264.

GAUR, R. and A. K. Pandey & D. K. Arora (1988). Effect of streptomycin on cellulase production by Trichoderma viride. Hindustan antibiotic. 30 (1 - 3) : 31-32.

GOEL, S. K. and R. S. Mehrotra. (1973). Production of pectolytic & celluloytic enzyme by Rizoctonia bataticola in vitro & viva. Indian Phytopathology. 27.171-177.

_______ (1973 - 1974). Effect of some fungicides including antibiotic on pectolytic & cellololytic enzyme activity of Rizoctonia bataticola. Hindustan antibiotics. 16(2-3) : 103 - 108.

_______ (1975). Production of Pectolytic & celluloytic enzyme of Bajra ergot fungus. Indian phytopathology. 28:123-125.

GOTTLIB, D. & R. K. Tripathi. (1968). The pysiology of swelling of spore gernination in Penicillium atroventum. Mycologia. 60: 571-590.

GREGORY, K. F. (1952). Antibiotics as agents for control of certain damping off fungi. Amer. J.Bot. 39:405-415.

GREGORY, P. H. (1961), The microbiology of the atmosphere. Lenord mill (Books) Ltd. London. 251.

GROVER, R. K. (1963). Effect of fungicides on pectolytic enzyme activity of fungi, current science. 9: 398-399.

_______ (1964). Effect of some fungicides on pectolytic enzyme activity of Sclerotinia and Botrylis allie. Phytopathology. 54 : 130-133.

GRUZDYEV, G. S., V. A. Zinchenke, V. A. Kalinin & R. I. Slavtrov. (1983). The chemical protection of plants. MIR Publishers Moscow. PP. 267 - 333.

GUPTA, S. R. (1976). The Ecological study of Themeda quadrivalvis type of grassland of Bundelkhand Region. P.H.D. thesis, Sagar University. Sagar.

GUPTA, S. & B. K. Gupta. (1984). A Study of bysinosis & associated respiratory disorder in cotton mill workers Delhi, Int. aero Soc.

GYLLENBURG, H. G. & Eva, Eklund. (1974). Bacteria. "In Biology of plant litter decomposition" eds. by C.H. Dickinson & G. J. F. Pugn Vol II : 245.

HANCOCK, J. G., R. L. Miller and J. W. P. Lorbeer. (1964). Phytopathalogy. 54 : 929-931.

HOGG, M. B. (1966). Microfungi on leaves of Fagussylvatica II duration of survival, spore viability & cellulolytic activity. Trans. Brit. Mycol. Soc. 49: 193-204.

HORSFALL, J. G. & S. Rich, (1953). Fungus Killers yale Sci. May. 27,12 cited by Horsfall, J.G. (1956) in Principal of fungicidal action., 280 PP. Walthman, Clronica Botanica Company.

HORSFALL, J. C. (1956). " Principle of fungicidal action chromica Botanica, Weltham, Mass.

HUSAIN, S. S., Begum, Firdousi, M. K. A.Khan & S. S. Anwar. (1971). Fungi toxicity of some benzofuram derivative to A. niger sind university Res. J.5 (2): 201-205.

JACKS, H. & H. C. smith, (1952). Soil disinfection, XII, effect of fumigants on the growth of soil fungi in culture. Newzealand. J.Sci. technol. Sect. A.33:69-73.

JAIPRAKASH, et. al., (1978). Aspergillus flavus in the air of working environment. current science. 47: 920-921.

JAIPRAKASH, and Ramalingan, (1981). Aspergillus fumigatus in air of working enviroment at Mysore. Indian J.Bot. 4: 17-25.

JACOBY, G. A. & L. Gorlani (1968). Effect of streptomycin & other antinoglycosid antibiotices I. Mecheanism of action edited by David Gottlieb & Paul. D. Showspinger verleg Berlin, Heidelbery- New York. P-726.

JENSEN, H. L. (1931). The microbiology of farming & manure decomposition in soil II. Decomposition of cellulase. J.Agric Sci. 21:81-100.

JOHNSON, L.F. and E.A. Curl. (1972). " Methods for Research on ecology of soil - borne plant pathogens." Burgess Minneapolis.

JOHNSON, L. F. (1957). Effect of antibiotic on the number of bacteria & fungi isolated from soil by dilution plate method. Phytopathology. 47(10):630-631.

JOHN, W. corcoran & fred F. Halm (1969). Antibiotic III. Mechanism of action of antimicrobial & antitumor agents. Spinger verleg Berlin, neidecl berg. New York.

KADIRVEL, U.R. and A. Mahadevan. (1967). Pectin polygalacturonase transeliminases in Fusarium moniliforme & Cephalosporium sacchari, Current science. 36:396.

KAARIK, A. A. (1974). Decomposition of Wood - Biology of Plant litter Decomposition. Eds. Ch. Dickinson & G. J. F. Pugh. Vol. I:129-174.

KAMAL, & Shrivastava. (1975). Succession of microfungi on decaying liver wort (Cyathodium sp). Proc. Nat Acad Sci. India. 45 (B)111 : 185-195.

KANNAIYAN, S. (1988). Effect of certain fungicides on the production of enzymes by Rizoctonia solani. Plant & Soil. 108(2),299-302.

KANNAIYAN, J. P. Vidyaseharan and T. K. Kandaswamy. (1975). Production of pectolytic & cellulolytic enzyme by Bajra ergot fungus. Indian Phytapath. Vol.28:123-125.

KARKHAN, R. S. (1969). M.Sc. (Vetery). Thesis submitted to A.P.Agricultured university.

KOOIMAN, P. (1957). Some properties of cellulase of Myrothecium Verrucaria & some other fungi II Engymologia. 18 (6) : 371-384.

KORZYBSIKI, T., Z. Kowszyr. Gindifee and W. Kuryeowicz. (1969). Antibiotics origin, nature & properties. Volume. II. 1146.

KUTHUBUTHEEN, A. J.and G. J. F. Pugh. (1979). The effect of fungicides on soil fungal population. soil Bioal. Biochemistry, 11(3):297-304.

LAGEERE, R. (1973). Bollrots of cotton in Senegal. II fungi isolated from ball during rot, cotton et. fibre Tropicals. 28(4):493-507.

LENNETTE, E. H., E. H. Spauding and J. P. Truant. (1974). Mannual of clinical microbiology 2nd. ed. American Society for microbilogy Washington.

LI, L. H. and R. M. Flora and K. W. King. (1965). Indivisual role of cellulase components derived from Trichoderma viridi. Arch. Biol. Chem. Biophys. II : 439 - 447.

LOCKWOOD, J. L. (1964). Soil fungistasis. Ann. Rev. Phytopath. 2 : 341 - 362.

LUCAS, R. L. (1955). A comparative study of Ophiobolus graminis and Fusarium culmorum in sparophytic colonization of wheat straw. Ann. Appl. Biol. 43 : 134 - 143.

LUSIS, AND Bcker. (1973). Glucosidases of Chaetomium thermophile var. Copraphile var. Biochem. Biophys. Acta. 329 : 5 - 16.

KANNAIYAN, J. P. Vidyaseharan and T. K. Kandaswamy. (1975). Production of pectolytic & cellulolytic enzyme by Bajra ergot fungus. Indian Phytapath. Vol.28:123-125.

KARKHAN, R. S. (1969). M.Sc. (Vetery). Thesis submitted to A.P.Agricultured university.

KOOIMAN, P. (1957). Some properties of cellulase of Myrothecium Verrucaria & some other fungi II Engymologia. 18 (6) : 371-384.

KORZYBSIKI, T., Z. Kowszyr. Gindifee and W. Kuryeowicz. (1969). Antibiotics origin, nature & properties. Volume. II. 1146.

KUTHUBUTHEEN, A. J.and G. J. F. Pugh. (1979). The effect of fungicides on soil fungal population. soil Bioal. Biochemistry, 11(3):297-304.

LAGEERE, R. (1973). Bollrots of cotton in Senegal. II fungi isolated from ball during rot, cotton et. fibre Tropicals. 28(4):493-507.

LENNETTE, E. H., E. H. Spauding and J. P. Truant. (1974). Mannual of clinical microbiology 2nd. ed. American Society for microbilogy Washington.

LI, L. H. and R. M. Flora and K. W. King. (1965). Indivisual role of cellulase components derived from Trichoderma viridi. Arch. Biol. Chem. Biophys. II : 439 - 447.

LOCKWOOD, J. L. (1964). Soil fungistasis. Ann. Rev. Phytopath. 2 : 341 - 362.

LUCAS, R. L. (1955). A comparative study of Ophiobolus graminis and Fusarium culmorum in sparophytic colonization of wheat straw. Ann. Appl. Biol. 43 : 134 - 143.

LUSIS, AND Bcker. (1973). Glucosidases of Chaetomium thermophile var. Copraphile var. Biochem. Biophys. Acta. 329 : 5 - 16.

MACER, R.C.F. (1961) Saprophytic colonization of wheetstraw by **Cercosporella herpotrichioidis** from other fungi. Arn Appl Biol. 49: 152-163.

MALATI, Mazumdar, D.Som. (1987). Evalution of some systamic & non systamic fungicides against pathogens of blast fiber crop. pesticides. 29(9):33-36 (Div.Mycol.pl.Path.Jute.Agric.Res: Inst. Barackkore) Pin.743101.India.

MALIN, E. (1948). Recent advances in the study of tree mycorrhiza. Trans.Br.mycol. soc.30:92-99.

MARSH, P.B.& Bollen bacher. K. (1949). The fungi concerned in fiber deterioration Part I : Their occurence. Taxtile Res. J.19:313-324.

MARTIN, J.P. (1950). Uses of acid, rose Bengal & streptomycin in the plate method for estimating soil fungi. Soil Sci. 69:215-232.

MAXON, E.Simpson & Paul B.Marsh. (1969). Microscopic observation on fungi associated with cotton Boll - rot fibers. Mycologia.61:P.987.

MEHTA, P.R.. (1971). Role of fungicides in modernizing agricultur in India . Eigth Mondkar Memorial Lecture at the annual meeting of Indian Phytopath Soc. at Bangalore.

__________ (1980). Inter relationship between **Caprinus lagopus** & **Fusarium udum** in soil in relation to competitive saprophytic ability and microbial antagonism. Plant and soil . 56 : 169-173.

MEHTA, P. (1974) Inhibitory effect of some fungicides on cellulose Production by **Alternaria sp.**. Hindustan Antibiotics.17(1):12-15.

MEHTA, A .& P. Mehta. (1988). Effect of Antibiotics on Synthasis of cell wall degrading enzymes. By. **Furavium oxysporum** & **Fusarium moniliforme**. Hindustan Antibiotics. Vol. 30 No.(1-2):27-30.

MEHTA, S.K.& R.S.SANDU (1983). Immulogical significance of **Aspergillus fumigatus** in cane sugar mill. 38:41-46.

MEHROTRA, R.S.& K.R.Aneja (1979) Microbial decomposition of **chenopodium album** litter I succession of decomposition. Jour Indian Bot.Soc.58:189-196.

MEHROTRA,R.S.(1980). "Plant Pathology".Tata Mcgraw hill publishing company Ltd. New Delhi.PF-279.

MENDELS,M & E.T.Reese(1965)Inhibition of cellulose Ann. Rev. Phytopatho. 3:85-102.

MENDELS, M & J.Waber.(1969).The Production of cellulose Advance chem. 95:391-414

MICHAEL,J., Pelwar. J.R.,E.C.S. CHAN. Noel R. Krieg. (1977). "Microbiology". Control by chemical.

MOHENDRANATH,J.,A.Sushagiri Rao,K.Prabhudas,B.Dhanap Jaya Reddy & V. N.Pargaanker. (1976). Study of Mastitis with special refrence to antibiotics senstivity. Vol.16.(3):116-119.

MUTHAL,A.K.& S.B.Saxena(1973).Production of cellulase by Fusarium, dimorumn Penzi Causal organism of fruit rot of Luffa .Proc.Nat. Acad. Sci. India. 43(B).I&II.

NANCY,L.Oleinick.(1969). Erythromycin sensitivity of selected Bacterial organism.Antibiotic III. P.396. Spinger verley Berlin, Heidelbery, New York.

NEWSHELL,A.G. (1955). Disinfestation of soil by hast, flooding & fumigation Bot. Rev. 21:189-250.

NIGAM,S.S.,S.K. Ranganathan, S.R. Sengupta, R.K.Shukla, & R.N.Tandan,R.N.(1959).Microbiol degradation of cotton cellulose in soil J.Sci. Induster Res. 19.(C):20-24.

NORKANS,B.(1950b).Sybolial Botan opsoliensis,11(1):1-126.

a? Where is

NORKANS,B.&.B.G. Ranby. (1956). Studies of enzymatic degardation of cellulose, Physiol Plantarum. 9:198-211.

NORKANS,B.(1963). Degradation of cellulose. Ann. Rev. phytopath. 1:325-350.

a? b?

NORKANS,B.(1963).Influence of some cult condition on fungal cellulose production. Physiologia plantarum. 16:11-19.

NORKANS,B & A.Hammarstrom .(1963). Studies on growth of Rhizina underlata Fr. & its production of cellulase & pectin-decomposing enzymes. physiol. plant. 16:1-10.

NORMAN,A.G.& W.H.fuller.(1942). cellulase decomposing by micro-organism.Advance in Enzymology .2:239.

OSO,B.A.(1978).The production of cellulase by Talaromyces emersonii. Mycologia.71:520-535.

OWENS,R.G.(1963). Studies on the nature of fungicide action, Inhibition of dulphydryl amonoiron & copper dipendent enzymes in vitro by fungicides & related compound. Contrib, Boycl. Thompson Inst:20: 476-497.

PANDAY,P.N.(1980). studies in soil microbiology with special refrence to litter decomposition Ph.D. this is, Sagar University, Sagar.

PARK ,D.(1958) The saprophyrtic status of Fusarium oxysporum Schl. causing vascular wilt of oil plan. Ann. Bot. tond. 22:19-35.

PARK,D.(1959). Some aspects of biology of Fusarium oxysporum Schl.in soil.Ann.Bot.Lond.23:25-49.

PARKINSON ,D.,T.R.G.Gray & S.T.Williams.(1971)."Methods of studing the ecology of soil micro-organism. Black wall-oxford.

PARTRIDGE,A.D.& A.E. Rich .(1962).Induced tolerance to fungicides in three Sp. of fungi, Phytopathology. 52: 1000-4.

PARRY,K.E.& R.K.S.Wood.(1958). The adaptation of fungi to fungicides. Adaptation to copper & murcury salts. Annual. Appl. biol. 46:446-456.

PARRY,K.E.& R.K.S.Wood.(1959).The adaptation of fungi to fungicides. Adaptation to captan. Annual.Appl. Biol.47:1-9.

PARRY,K.E.& R.K.S.Wood(1959).The adaptation of fungi to fungicides,Adaptation to Thiram, Ziram,Ferbem, Nebam, & Zineb. Ann.Appl. Biol.47:10-16.

PATIL,A.S.Rao,V.G.(1972). In vitro effect of certain fungicides & Antibiotics on germination & mycilial growth of Two phytopathegenic fungi (Alternanria alternata, Phyllosticta gerbericola).Hindustan Antibiotic Bultetin. 1972.15(1/2):34-39.

PALEZAR.J.M.(Jr.)Roger, D. Reid & E.C.S. Chan. (1977). Antibiotic & other chemotherapeutic agents. 466-493. Microbiology Tata McGrow Hill New Delhi.

POLYAKOV,I.M. V.I.Popov & M.Y.A.Menlikev (1968).chemical, cmmunizasion as a mean of controlling Fusarium wilt of thin fiber cotton, Khimiya Sel, Khoz, 6(12):30-33.

POWER,C.B.& H.F. Daginawala. (1982). Antibiotics 386-420. General Microbiology Vol.II Himalaya Pub. House Bombay.

PRINGSHEIM ,H.(1912). The Enzymatic break down of cellulase. Ziet Physiol - chem. 78:266-291.

PUGH,G.J.E.(1958).leaf litter fungi found on Carix Paniculata. Trans.Britl.Mycol. col.Soc.45:567-572.

PUGH,G.J.E.(1966).Cellulose decomposing fungi isolated from soil near Madras. Jour Indian Bot. Soc. XLV.3-4; 232-241.

QUESHEL.Barbara,L.Scott & Janel Taylor(1971).the effect of Dimethyl sulphoride (DMSO) on the survival & germination of baccillus substillis spores. Spore Research By Barker gould wolf. P.P.303.

RAI,B.(1969). Comparative cellulolytic ability of some fungi. Anabstract in Proc. 56th Indian Sci. Cong. Part III.P.314.

RAI,B.(1970). Comparative cellulolytic abiity of some fungi. Lab. Dev. Jour. of Sci.& technology, Kanpur,(India). B.8:251-252.

RAI,& Shrivastava. (1982). - Decomposition of leaf litter in relation to Microbial population & their activity in a tropical dry mixed deciduous forest.Pedobiologia. 24(3):151-159.

RAM RAJ ,B. & P. Vidhya shekharn (1986). Inactivation of pectic enzymes produced by Phytophthora parasitica VAR piperina by fungicides. Indian phytopath.39(2):269-270.

RAO,A.S.(1958).Acomparision of saprophytic behaviour in various root infecting fungi. Ph.D.thesis, University of cambridge.

RAO, A.S. (1959) . A comparative study of competition saprophytic ability in twelve root-infecting fungi by an agar plate methods. Trans-Bril-Mycol Sec. 42(1):97-111.

RATI, & Ramalinagam.(1976).Air borne Aspergilli Mysore. Aspects of allergy & Appl. immuno.9:139-49

RAUNKIGER, C.(1934).The life form of plants & statistical plant Geography being the collected papers of C.Raunkiaer clarendon press, Oxford, England.

RAYNAL, G.(1973).Aspergillus niger V. Tiegh, **Rhizopus stolonifer** :Lind & two agents of stigmatomycosis responsible for the coloration cotton fibre in Iran. Cotton Fibres. Tropicals.28(2):297-300.

REESE ,E.T.(1946).Aerobic decomposition of cellulose micro organism at temperature above 40 degree C (unpublished Ph. D. Thesis, Pennsylvania state college eveston, Penn) Quoted by cooney & Emerson (1964).

REESE, E.T.(1947).On the effect of aeration & nutrition on cellulose decomposition by certain bacteria J.Bact. 53:389-400 .

REESE ,E.T., R.G.H. SIU & H.S. Lavinson. (1950). The biological degradation of soluble cellulose derivatives & its relationship to the mechanism of cellulose hydrolysis . Jour. Bacterial. 59:485.

REESE ,E.T.& H.S.Livinson. (1952).A comperative study of the break down of cellulose by micro-organisms. Physiologia PL.5:345-366.

REESE, E.T.& Segal & Tripp. (1957). Text. Res. Jour. 27:626. Title

REESE, E.T. (1963). Advance in the enzymatic hydrolysis of cellulose related materials. Pergamon Press, New York & London.

RICH, (1960).'Fungicidal chemistry' in plant pathology . J.G. Horsfall & A.E.Dimond(Eds.)Academic press New York & London .III.553-603.

ROHANLKER P.W. & P.Neergaard (1969). Studies on aureo fungin as sead treatment in controlling sead borne fungal diseases. Hindustan antibiotic Bull. 11:163-165.

RUSENBERG, S.L. (1978) . Cellulose and ligno cellulose degradation by thermophilic & thermotolerant fungi. Mycologia. 70:1-11.

SADASIVAN ,T.S.(1939). Succession of fungi decomposing wheat straw in diffrent soil with spacial reference to Fusarium culmorum. Ann. appl. Biol. 26.497-508.

SANTRA,& Chandra (1981)Indoor airborne fungal spores flora of calcutta west Bangal. Proe. Nat. Cong. env. Biol. 45-48.

SBRAGIA.K.L.(1975). Chemical control of plant diseases. An exciting future.Ann.Rev.Phytopath,13:257-269.

SELBY,K.(1961).The degradtion of cotton cellulose by extra cellular cellulose of mycrotherium verrucaria. Biochem. J.79:562-66.

SELBY, K. & C. C. Maitland . (1967) . The cellulose of Trichoderma viridi Separation of the components involved in solubilization of cotton.Biochem.J.109:716-724.

SETHI,Alka. (1991). Microfloral Decomposition of some organic urban waste. Thesis submitted to university of Bundelkhand.

SHANKARNARAYAN,K.A. & P.M. Dabadghao.(1970).Ecological studies on the grassland of Jhansi. U. P. Ann. Aridzone 9:54-70.

SHALTLA,M.N. & J.B. Sinclair,(1963). J.B. Tolerance of Pentachloronitrobenzene among cotton isoletes of Rizoctonia solani. Phytopathology. 53:1407-11.

SHARMA ,R.B. & Ray A.N. (1979). Boll rot of cotton. curr. sci. 48:413-414.

SHARMA ,B.K. & M.S.Chauhan .(1985). Lab evaluation of fungi toxicant & antibiotic against four foliar Cotton pathogen. Agric-Sci.digest. 5(3)125-128.

SHUKLA,R.K. & R.N.Tandon.(1960).Microbial degradation of cotton cellulose in soil.J.Sci. Induster Res. 19.C:20-24.

SIMBYABUNYA,M. & Boyle.Alice M.(1969). Cotton boil root in Arizone, Phytopathology. 59(5):667-668.

SIMPSON MARION,E. & P. B. Marsh. (1969). Microscopic observation of fungi associated with cotton boll rot fibre.Mycologia.61(5):987-996.

SINDHU,M.S.& D.K.Sandhu.(1974).cellulolytic enzyme of seven fungi isolated from decompossing sugar cane Bogasse. Indian. Jour, of microbiology. 194:204-205.

SINGH, K. P, M. K. Sinha, S. C. Tripathi & J.C. Edward. (1973). Invitro effect of diffrent concentration of Aureofungin on the inhibition of spores germination of five Alternaria:species.Hindustan Antibiotic Vol. 15. No. 4: PP. 154-155.

SINHA ,M.K.,R.Jeyarajan & S.P.Kapoor.(1972).Fungicides of control of Pre harvest fruit drop in sweet orange. Indian phytopathol. 25:262-265.

SINHA, et.al.,(1981).Aeromycology of calcutta, Aspects of allergy & Appl. immunol.14:67-71.

SIU,R.G.H. (1951). Microbial decomposition of cellulose (Reinhold Publication Corp. New York)

SIU,R.G.H. & E.T. Reese. (1953). Bot Rev. 19 :377. Title

SRIDHAR ,T.S.(1974). Evaluation of fungicides against **Rhizopus sp.** causing soft root of fruit. Hindustan antibiotic.

SMIT,J.& K. T. Wieringa, (1953). Microbiological decomposition of litter. Nature, Land. 771-794.

STERNBERG,D.P.Vijay Kumar & E.T.Reese.(1977).Can.J.of Microbioilogy. 23:139-147. Title

SUGUNAKARA, Readdy, M.S.Rama Nandu and A.Appa Rao.)(1979). Factors effecting fungicides tolerance by certain fungi, in vitra. The Andra Agric. J.26(5 & 6):197-199.

TEWARI, Rashmi. (1991). Fungi in the indoor air of saw mills in Lucknow.and their role as allergenes. Thesis sumitted to university of Kanpur.

THAKRE ,R.P. & B. N. Johri. (1973). Invitro activity of Aureofungin Amphotiricin B & Hamcyin on the mycelial growth of some thermophilic fungi. Hindustan Antibiotics. Bull. 15(3):75-78.

THAKRE ,R.P. & B. N. Johri. (1973- 74). Influence of antibioitics on swelling phase & spore germination of thermophilic fungi. Hindustan Antibiotic Bull. Vol. 16. No. 2-3:109-114.

THRIUMALACHAR, M.J., P.W.Rahlkar, R.S.Sukepure & K.S.Gopal Krishnan. (1964). Aureofungin a new heptaene antibiotic part I. Microbiological studies. Hindustan antibiotic Bull. 6:108-111.

THIRUMALACHAR, M.J. (1968). Antibiotics in the control of plant pathogens.Adv.Appl.Microbiol. 10:313-337.

TILAK and Chakre . (1974). Atmospheric connectration ?

Tilak and Chakre, (1979). Atmospheric concentration of Claviceps in relation enviroment factors. IV, International plan.conf.LUCKNOW, PP.181-182

TRIVADI, B.K. (1975). Ecological studies of some grassland of central India. Ph.d.Thesis Sagar Univirsity Sagar.

TRIBE, H.T. (1957). Ecology of Micro organism in soil as observed during their development upon buried cellulose film. Symp. Soc. gen. microbial. 7:287-298.

---------- (1960). Decomposition of buried cellulose film with special reference to the ecology of certain soil fungi . In the ecology of soil fungi, ed.D.Parkinson & J.S. waid liverpool university press. ?

TROGESON, D.C. (Ed). (1967). Fungicidis an advanced. acadamic press New York & London. Vol. I: P.669.

UPADHYAY, R.S. and B.Rai (1982). Ecology of Fusarium udum causing wilt disease of pigeon pea, population dynamic in the root region. Trans. British Mycol. 50.C.78:209-220.

VAN,Iter son,G.(1904). Die. Zerseluzong Von. cellulose durch aerobe microorganism Zentable. Bakt. Parasit Kde. Abst.II, 11 :689-698.

WAKSMAN,S.A.(1927).Principles of soil microbiology williams & williams Co. Baltimore Md. PP.897.

WAKSMAN.S.A.(1952).Soil Microbiology . John. wiley. New york. 356.PP.

WALSH, J.H.and C.S.Stewart.(1971).Effect of Temperature, Oxygen & carbonpdioxide on cellulolytic activity of some fungi . Trans Brit. Mycol. Soc. 56:75-84.

WANG,S.C.and Pinckard, J.A.(1971). Pectic enzymes produced by Diplodia gossypina in vitro and in infected cotton bolls. Phytopathology. 61(9):1118 - 1124.

WARCAP,J.H.(1960) Methodes of isolation and estimation of activity of fungi in soil. In "The Ecology of soil fungi"Ed. D.,Parkinson . & J.S.Waid Liverpool. University Press.

WASTIE. R.L. (1961).Factors effecting competitive, saprophytic colonization of agar plate by various root infecting fungi. Trans; Brit. Myco.Soc.44:145-154.

WEBLEY,D.M.and R.S.Duff. (1962). A technique for investigating localized. mocrobiol. development in soil. Nature. London. 192.364-365.

WLRITAKAR,D.R. (1971). Celluloses. In P. D.Bayer,ed.The enzymes acadamic press New York. 5:273-290.

WHITE,W.L.R.T.Darby.G.M.Steehert and K.Sanderson. (1948).Assay of cellulolytic activity of molds isolated from fabrices & related items exposed in tropies. Mycologia.40:34-84

WOOD,R.K.S.(1960).Pectic and cellulolytic enzymes in Plant diseas. An. Rev. Plant Physiology.

WOOD,R.K.S.(1955). Pectic Enzymes secreated by pathogens and their role in plant infaction. In J.W.Howic and A.J.O. Hea (eds) Machinizm of microbiol pathologenicity 5th symposium.Soc. Gen .Microbiol, Landon cambrige university press Londan.263-293.

WOOD,T.M. & S.I.Mcrae . (1972). The purification and properties of C, Component of *Trichoderma koninghil.* Bio cham. J.128:1183-1192.

YAMANE,K.H.Suzue, K.Yanaguchi, M,Tisukada and K, Misizawa(1965). J. Firm. Technol. Japan. 43:271

YOUATTG.(1958).Fungal cellulose IX.,Growth of *tachybotrysatra* on cellulose and production of a B- glucosidase hydrolysing cellobiose. Australian, J.Biol . Science 11 : 209-217.

2 Stealy

APPENDIX - I

Media used.

1. Potato dextrose agar (Johnson *et. al.*, 1959, P.143)
2. Potato dextrose broth - agar not added.
3. Peptone dextrose agar with Rose bengal and Streptomycin (Johnson *et. al.*, 1959).
4. Czapek's dox yeast extract agar (Johnson *et. al.*, 1959).
5. Czapek's dox agar (Rao, 1959).
6. Soil extract agar (Allen, 1957).
7. Glucose Asparagine agar (Waksman, 1950).
8. Nutrient Agar (Waksman, 1961).
9. Nutrient broth (Waksman, 1961).
10. Blood Agar (Cruickshank *et. al.*, 1972).
11. Lowenstien Jensen media (Jensen 1955).

APPENDIX - II

IDENTIFICATION OF FUNGI:-

Fungi were identified as far as possible with the help of following manuals and monographs after studying morphological and cultural aspects in details.

(1) Barnett, H. L. 1960. Genera of Imperfect fungi.

(2) Ellis, M. B. 1971. Dematiaceous Hypomycetes.

(3) Ellis, M. B. 1976. More Dematiaceous Hypomyetes.

(4) Gilman, J. C. 1957. A manual of soil fungi.

(5) Raper, K. B. and C. Thom .1949. A manual of penicillia.

(6) Raper, K. B. and D.I. Fennell.1965. The genus Aspergillus.

(7) Subramaniam, C. V. 1971. Hypomycetes.

(8) Thom, C. and K. B. Raper 1945. A manual of Aspergilli.

(9) Tubaki, K. 1963. Taxonomic study of Hyphomycetes.

For indentification of bacteria following works were conusIted.

1. Bergey's Manual of Determinative bacteriology (1957).

2. Cruicshank et. al. (1975) Medical Microbiology.

3. Ananthanarayan, R. and C.K.Jayaram Panikar (1978). Text book of Microbiology.

4. Salle (1974). Fundamental principles of Bacteriology.

5. Power & Daginawala 1982. General Microbiology Vol. II

what ?

PLATE - I

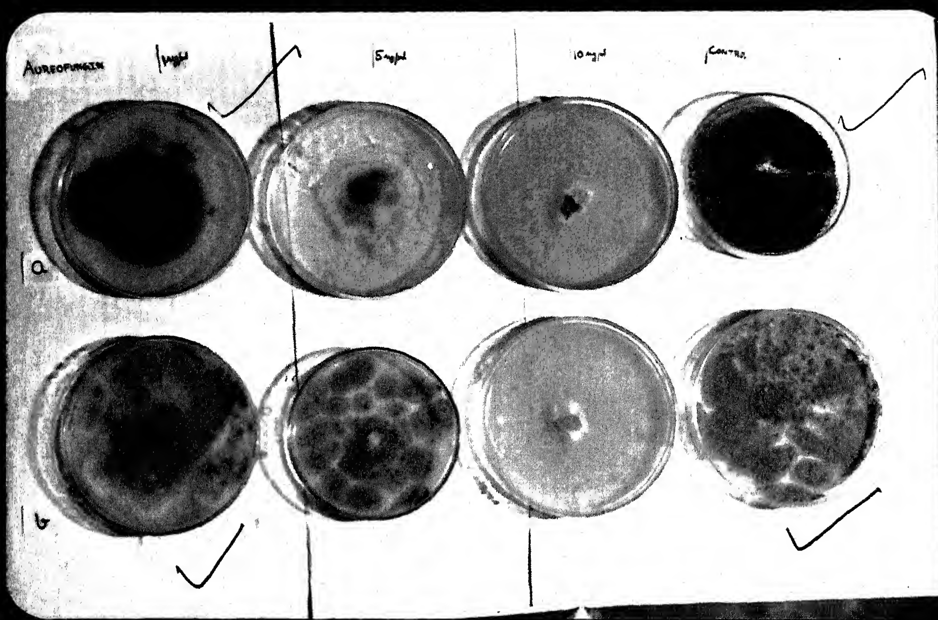
Minimum inhibitory concentration of Aureofungin against fungal test organisms by agar disc method.

a. Aspergillus niger.

b. Aspergillus flavus.

c. Penicillium citrinum.

d. Rhizopus nigricans.


AUREOFUNGIN
5 mg/ml
10 mg/ml
a
b

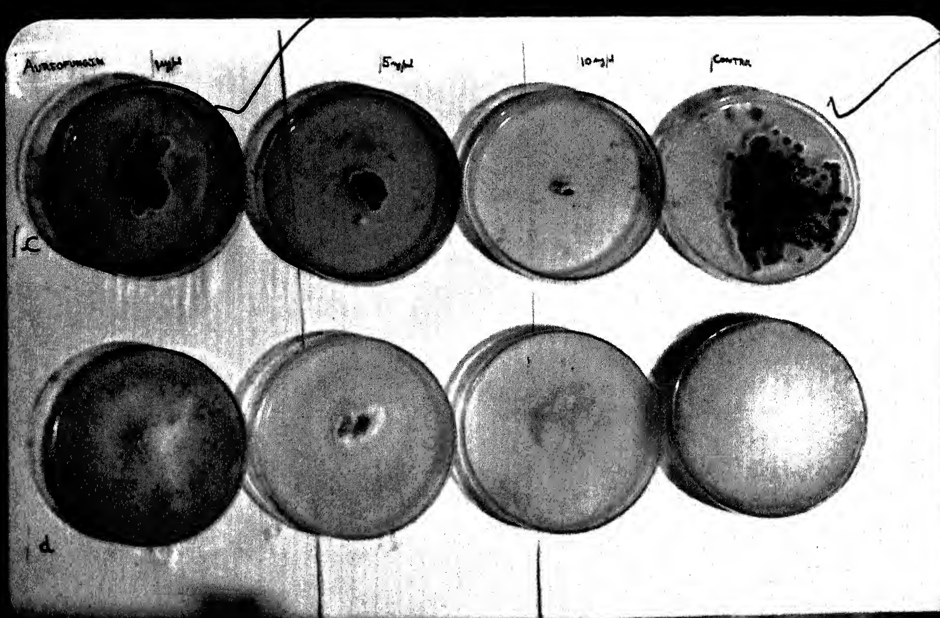

AUREOFUNGIN
5 mg/ml
10 mg/ml
c
d

PLATE - II

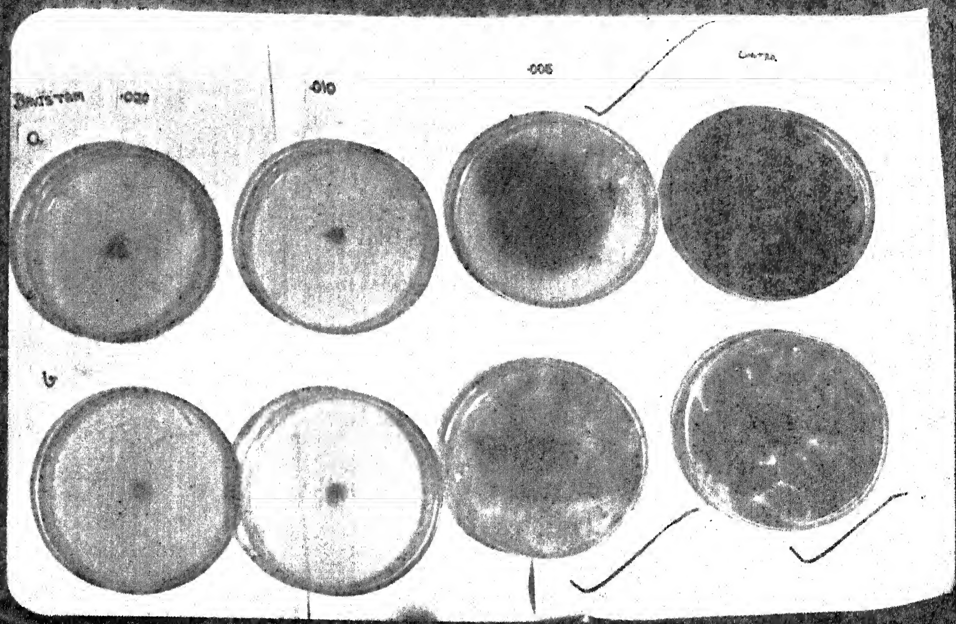
M.I.C. of Bavistin against fungal test organism by agar disc method.

a. *Aspergillus niger.*

b. *Aspergillus flavus.*

c. *Penicillium citrinum.*

d. *Rhizopus nigricans.*


·020
·010
·005
a
b

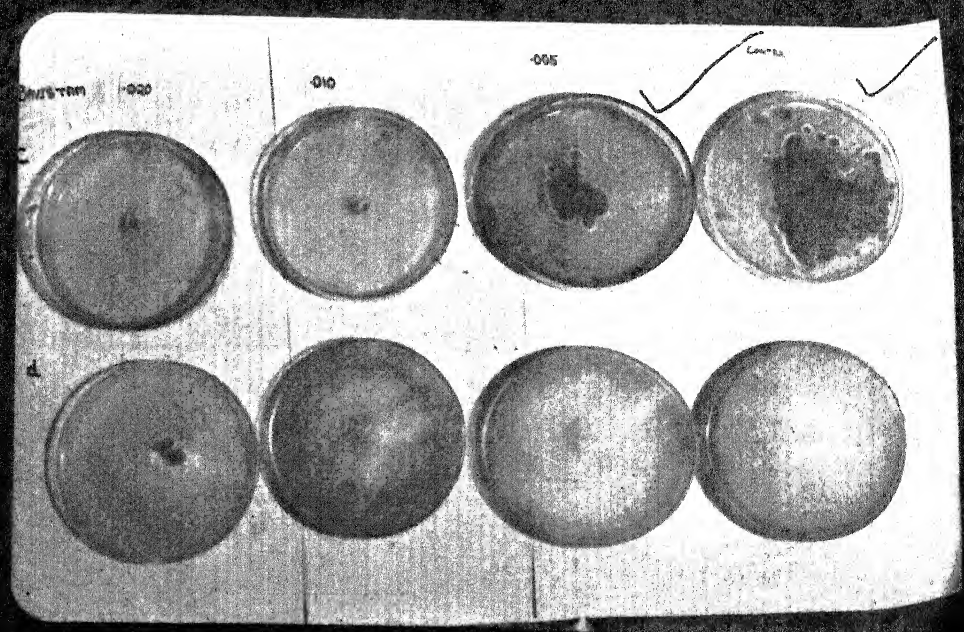

·020
·010
·005
c
d

PLATE - III

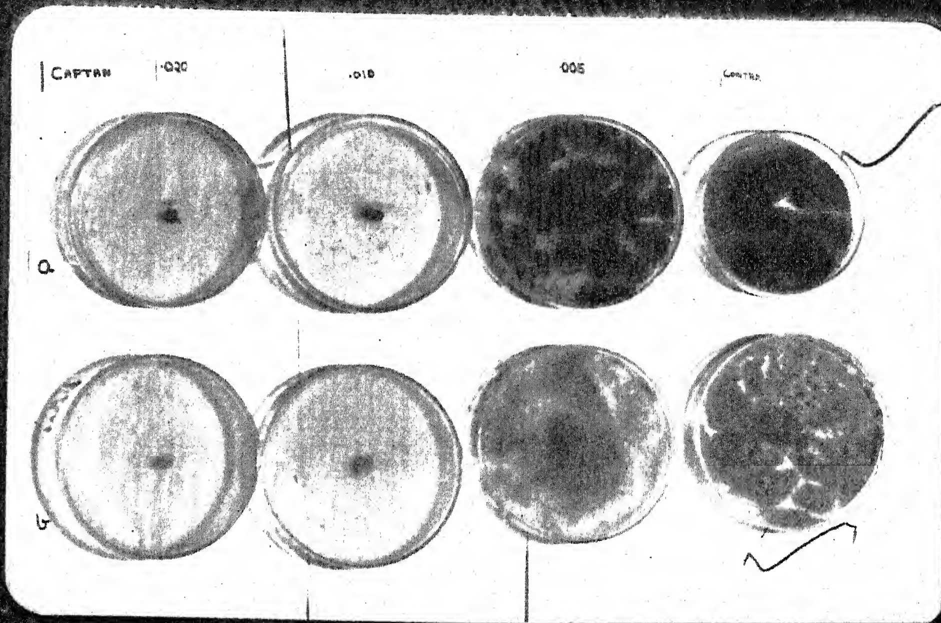
M.I.C. of captan against fungal test organisms by agar disc method.

a. Aspergillus niger.

b. Aspergillus flavus.

c. Penicillium citrinum.

d. Rhizopus nigricans.


CAPTAN
·020
·010
·005
a
b

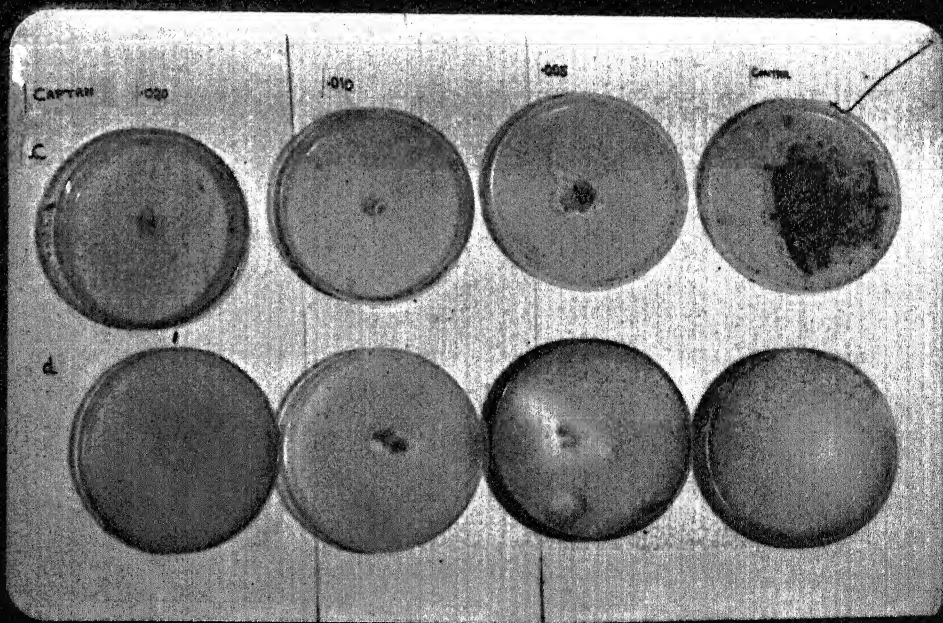
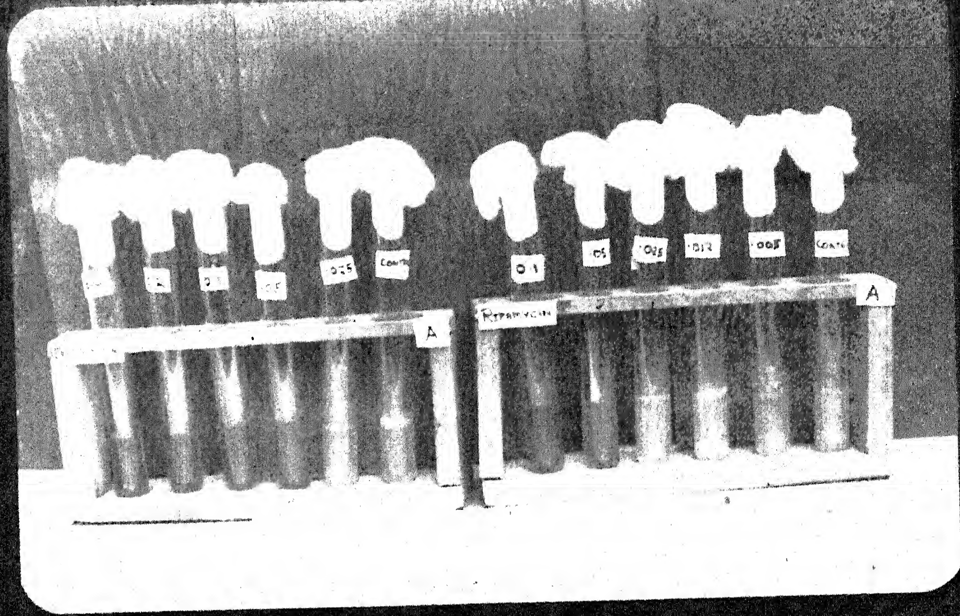
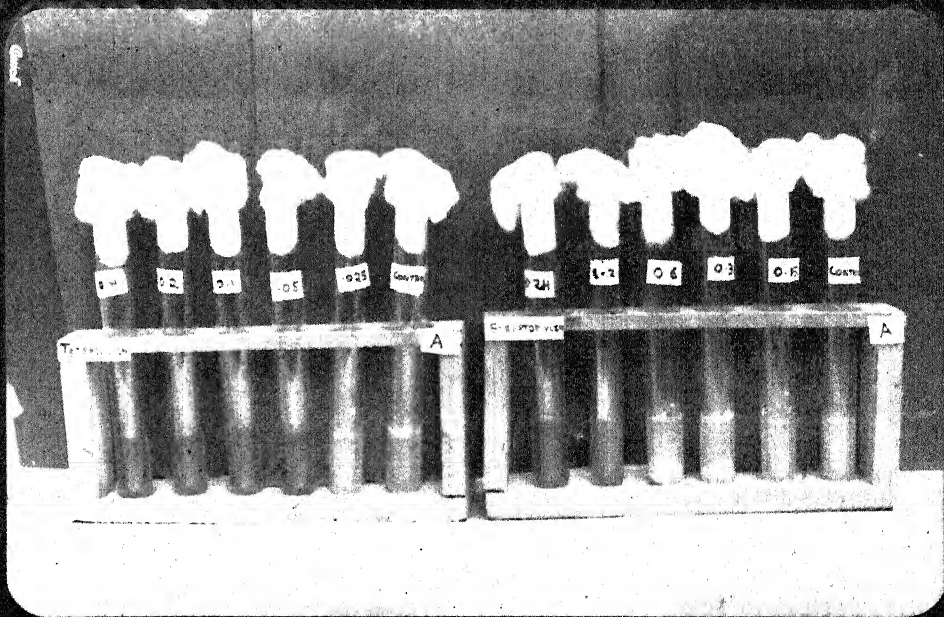

CAPTAN
·020
·010
·005
c
d

PLATE - IV

M.I.C. of antibiotics against bacterial test organism by tube dilution method.

a. Streptococcus pyogenes.


A
A


A
A

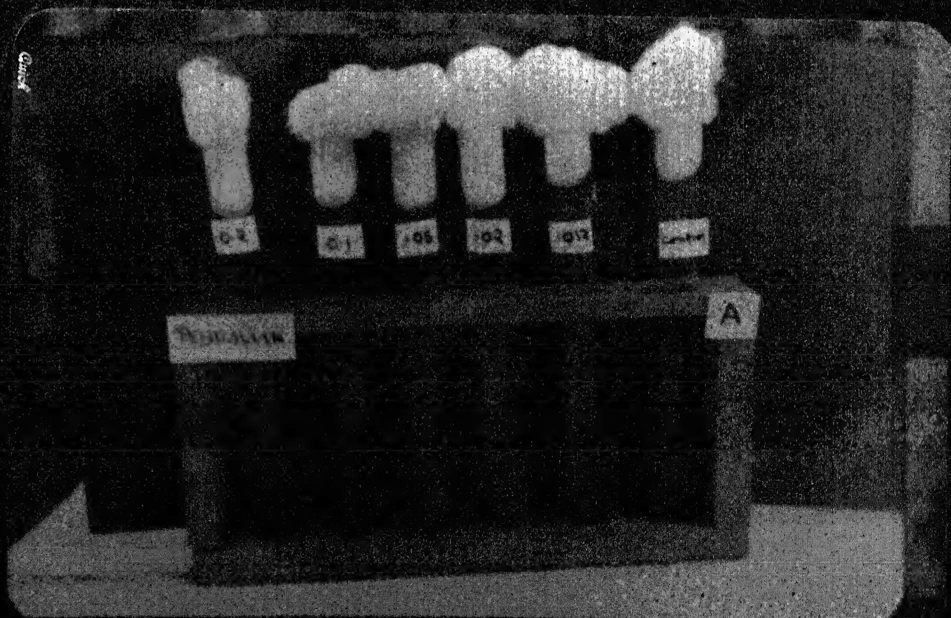
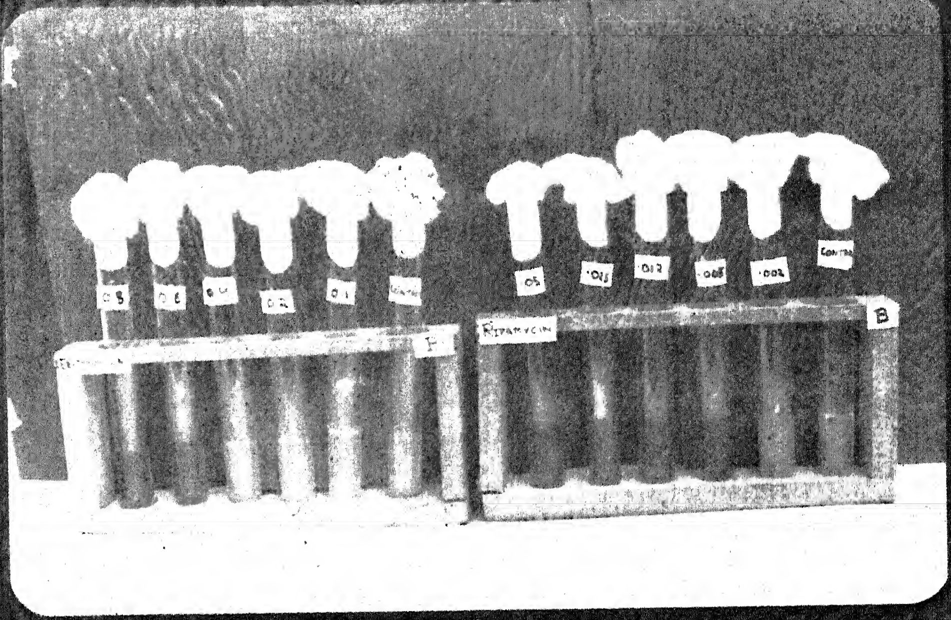
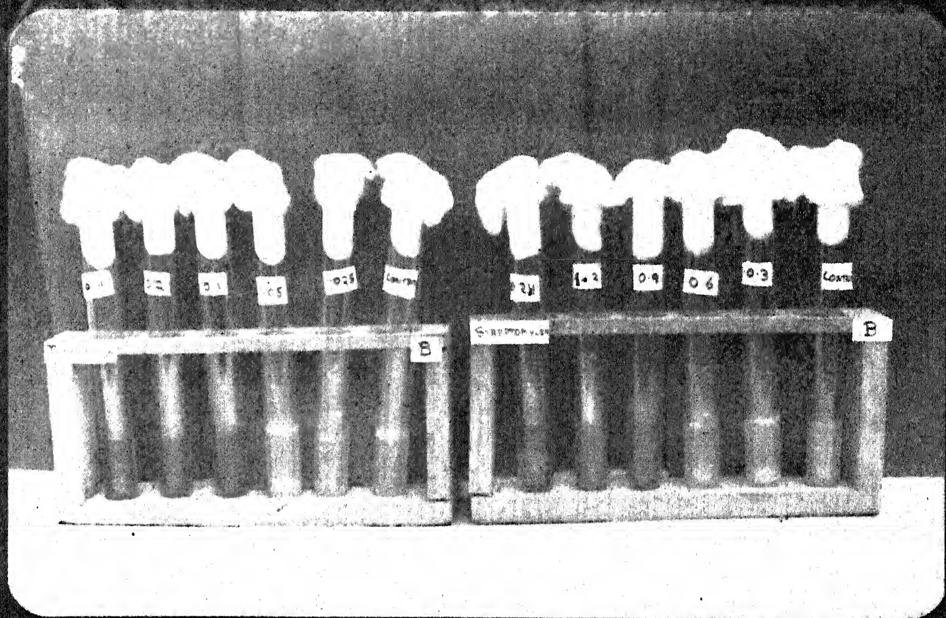

A

PLATE - V

M.I.C. of antibiotics against bacterial test organism by tube dilution method.

b. *Staphylococcus aureus*.


·05
·012
·002
B


·025
0·9
0·6
0·3
B
B

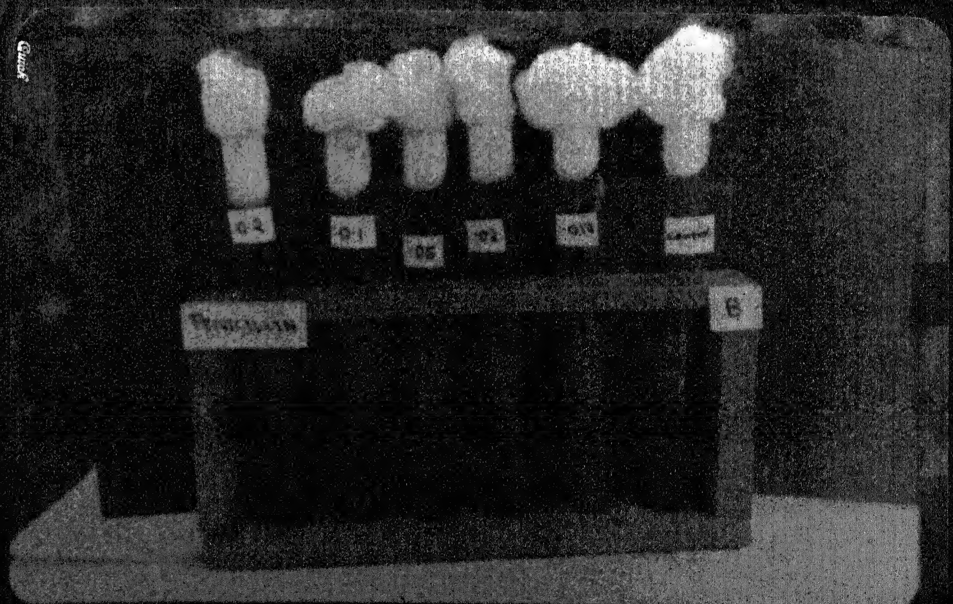
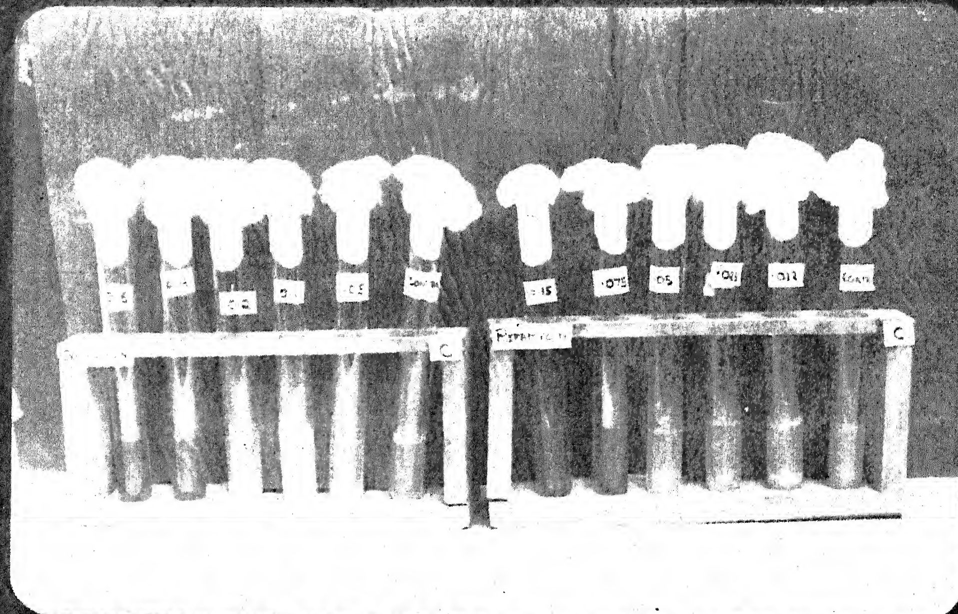
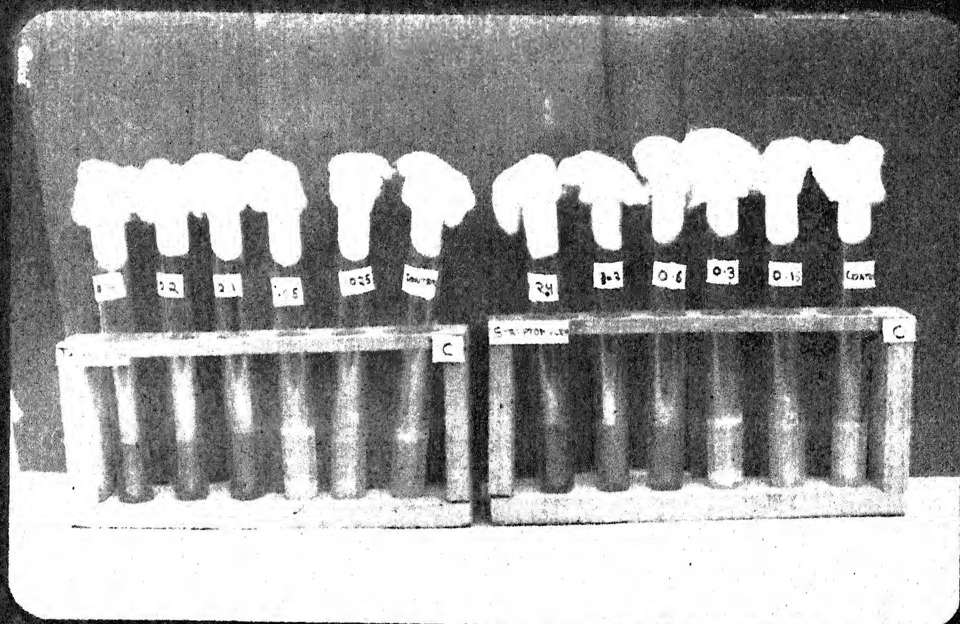

B

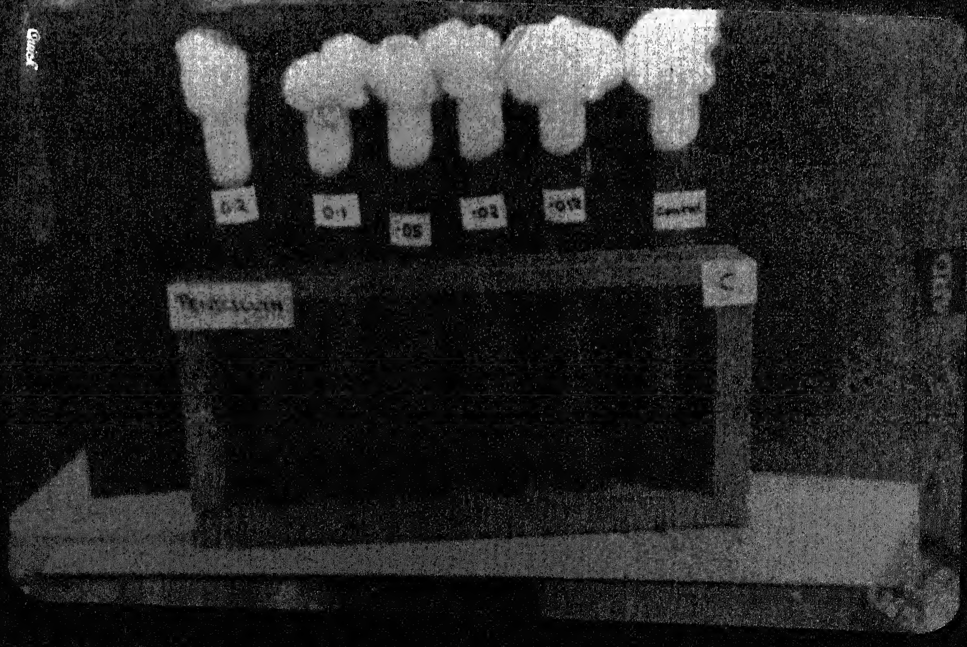
PLATE - VI

M.I.C. of antibiotics against bacterial test organism by tube dilution method.

c. Bacillus sp.






0.2
0.1
.05
.02
.012
Control
PENICILLIN
C